EVOLUTION AND POLLINATION

OF NEW ZEALAND *MYOSOTIS* (BORAGINACEAE)

A thesis

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by

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Myosotis australis var *conspicua*

Fog Peak, Canterbury.

Dedicated to David Lloyd,

whose enthusiasm for his subject is an inspiration to us all.

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ABSTRACT

The indigenous New Zealand species of *Myosotis* all belong to the southern section Exarrhena. The sister group to the southern species appears to be the discolor-group of eastern Africa and western Europe. It is postulated that the genus has an long history in New Zealand and is not the result of recent immigration from the northern hemisphere. In New Zealand, extensive speciation has occurred. Approximately 47 species are recognised. Radiation has proceeded in several different directions with respect to floral form. Several floral syndromes are recognised including tube-, funnel- and brush-blossoms. Some species have tended towards autogamy with a reduction in herkogamy, dichogamy, pollen:ovule ratio and petal size. Other species have become highly dependent on particular flower visitors including ants, beetles, flies and moths. All species are protogynous.

The pollination of one species, *M.colensoi* was investigated in detail. This species is self-compatible, but because of strong herkogamy requires a pollinator to deposit pollen on the stigma. It is visited primarily by a tachinid fly, *Protohystricia huttoni*. An investigation of the rates of pollen removal and deposition confirm that the female function is satisfied more quickly than the male. When conditions are unfavourable for pollination, however, there may be considerable overlap of the two functions. The number of flowers borne simultaneously on a plant of *M.colensoi* varies greatly. Larger plants receive more visits from *P.huttoni* and mature more seed. The visitation rate and male and female success per flower differs little between large and small plants, however. *P.huttoni* often visits many flowers at one bout on big plants suggesting that geitonogamous self-pollinations may be frequent. However, the proportion of flowers visited in one bout declines as the size of the display increases. This, coupled with a high level of pollen carryover, allows a considerable portion of the offspring to be the result of cross-pollination. It is suggested that other plants may be able to relieve some of the effects of geitonogamy by evolving features that increase pollen carryover. *P.huttoni* exhibits considerable directionality when moving over plants. It is suggested that such movement minimises the risk of encountering previously visited flowers.

CHAPTER ONE

AN INTRODUCTION TO THE SYSTEMATICS, BIOGEOGRAPHY AND FLORAL BIOLOGY OF *MYOSOTIS* IN NEW ZEALAND

This chapter serves as an introduction to the rest of the thesis and provides an overview of the systematic position of the New Zealand species of *Myosotis*. The systematics of the group is discussed in regard to the biogeography of the genus, and patterns of distribution within the new Zealand region are given. A synopsis of the recognised taxa is provided together with a scheme of tentative relationships. In the second part of the chapter, some introductory remarks on the floral biology of the New Zealand species are given. The pollination of 5 species is described and the nature of the relationships of the plant-pollinator interaction is discussed in the context of the New Zealand environment.

THE HISTORY OF MYOSOTIS IN THE SOUTHERN HEMISPHERE

In 1846 De Candolle (1846) produced a classification of the genus *Myosotis* including some Australasian species. He recognised 4 sections based on the presence or absence of corolla scales, the position of the anthers in the corolla tube, the ratios of the length of the anther to the length of the filaments and the presence or absence of an appendage at the base of the nutlets. This treatment has been largely accepted by flora writers since then including Gurke in Engler's and Prantl's Naturliche Pflanzenfamilien (Gurke 1897) and Hooker and Bentham (1876). The sections of De Candolle and their distinguishing features are:

Section I Eumyosotis D.C.
Corolla tube with small, often coloured scales; anthers upright, longer than the filaments, not exceeding the corolla tube.

Section II Exarrhena D.C.
Corolla tube with scales; anthers shorter than the filaments, exceeding the corolla tube.

Section III Gymnomyosotis D.C.
Corolla tube without scales, anthers shorter than the filaments exserted beyond the corolla scales

Section IV Strophiostoma Endl.
Corolla tube with scales, stamens not exceeding the corolla scales, the attachment region of the nutlets with a prominent caruncula.

In De Candolle's treatment the section Exarrhena was based on Robert Brown's genus *Exarrhena* which described a single species *Exarrhena suaveolens* from Australia. The section also included the species *M. staminea* (*synonymous with M.australis*? (Stroh 1941)), also from Australia. Two New Zealand species, *M. australis* and *M.forsteri* were placed in the section Eumyosotis. A third New Zealand species *M.spathulata* was placed in the section Gymnomyosotis apparently because of the mistaken impression that this species lacked corolla scales. De Candolle's fourth section Strophiostoma has been rejected as artificial (Grau and Liens 1968).

The last revision of the whole genus to appear was by Stroh (1941). In this treatment, the sections of De Candolle were retained and many other species added into the classification. The extra species treated included 30 New Zealand taxa and 1 species each from New Guinea and South America. *M.spathulata* was placed in section Exarrhena. In the Flora of New Zealand, Moore (1961) also adopted the traditional view of the genus, and recognised the two sections Myosotis and Exarrhena in New Zealand. In doing so however, she admitted at the time that

> "though the position of the anthers in relation to corolla scales is used in the first division of the key below, there is no firm conviction that this leads to the most nearly natural arrangement " (p 807).

Despite this recognition, she persisted with the classification and it is my opinion that this led her to recognising species on the basis of their relative anther exsertions that otherwise had little reason to be differentiated. Examples of this practise include her persistence with the species *M.venosa* as distinct from *M.forsteri* and her erection of a new species *M.elderi* as distinct from *M.lyallii*. In both cases, the only character that readily distinguishes the two species is the degree of anther exsertion.

Of *M.forsteri* she notes that of

> "plants ..... collected ..... on many North Island mountains, (the) relative lengths of filaments, of anthers and of styles vary ..... tending to bridge the gap between this sp. and *M.venosa*" (p 822)

and of *M.venosa*:

> "This species .... more closely resembles *M.forsteri*. Without the corolla and stamens specimens can be determined only tentatively by the slightly finer and more spreading hairs and the long style with clavate stigma" (p 823)

On page 832 in her notes on *M.lyallii* (section Exarrhena) she notes:

> "A few plants from the west coast of the South Id in BD herbarium closely resemble *M.lyallii* except that the anthers are more than half below the corolla-scales"

and similarly on page 812 in the notes describing *M.glabrescens*:

> "A special point of interest lies in the position of the anthers which does not place the sp. neatly in either section of the genus".

Clearly the New Zealand species do not fit easily into a subdivision of the genus into the two formal sections that have been used hitherto. As suggested by Moore, a classification based on the exsertion of the anthers is not natural. Grau and Liens (1968) also expressed doubts about the usefulness of De Candolle's sections. They pointed out that one European species regularly has exserted anthers despite being assigned to section Myosotis. In order to understand the evolutionary pathways of *Myosotis*, a more natural arrangement is needed.

Grau and his colleagues from Munich have made an excellent beginning in revising the genus (Grau and Liens 1968, Grau and Schwab 1982). In a study of the pollen morphology of the genus, Grau and Liens (1968) recognised that a basic dichotomy of the genus exists between the southern and the northern species and that the *discolor* group of annual species comprise a third somewhat intermediate group. Grau and Schwab (1982) followed this up with a study of several other characters of the pollen, stigma, corolla scales, and anther tips. They confirmed the distinctiveness of the northern and southern elements. The *discolor* group was again recognised as being somewhat intermediate. They proposed that a new infrageneric classification was

warranted to recognise this distinction, and they split the genus into two sections - Exarrhena and Myosotis. Although they have the same names, their sections are quite different from the former sections of De Candolle. In particular, all the southern taxa with the exception of the South African species are transferred into the section Exarrhena. Of the northern species, all except the species of the *discolor* group are placed into section Myosotis. The *discolor* group is placed as a sub-group in the otherwise completely austral section Exarrhena. Such an arrangement seems sensible on biogeographic grounds as well as taxonomic ones. It has strong implications concerning the origins of the southern group (see below) and allows us to consider evolution within the largely New Zealand section Exarrhena in a southern context. The distribution of Grau and Schwab's new sections is shown in Figure 1.

Synopsis of the Genus with emphasis on the Southern species.

A tentative synopsis of the species of *Myosotis* is presented here, taking into consideration the findings of Grau and his colleagues (Grau and Liens 1968, Grau and Schwab 1982). The taxa in the section Exarrhena that the present author recognises are indicated. This synposis may be compared with that of Moore (1961). The most important differences are the abandonment of the previous sections of De Candolle, which are replaced by Grau and colleague's new sections. Also, the basic dichotomy recognised within section Exarrhena involves the architecture of the stem. The classification is the result of several unpublished investigations of gross morphology, as well as detailed studies of the pollen and seed morphology. The list is provisional and may in some cases recognise groups based on plesiomorphic character states. Accordingly it should not be regarded as a "phylogeny", simply as a first attempt to order the species in some way.

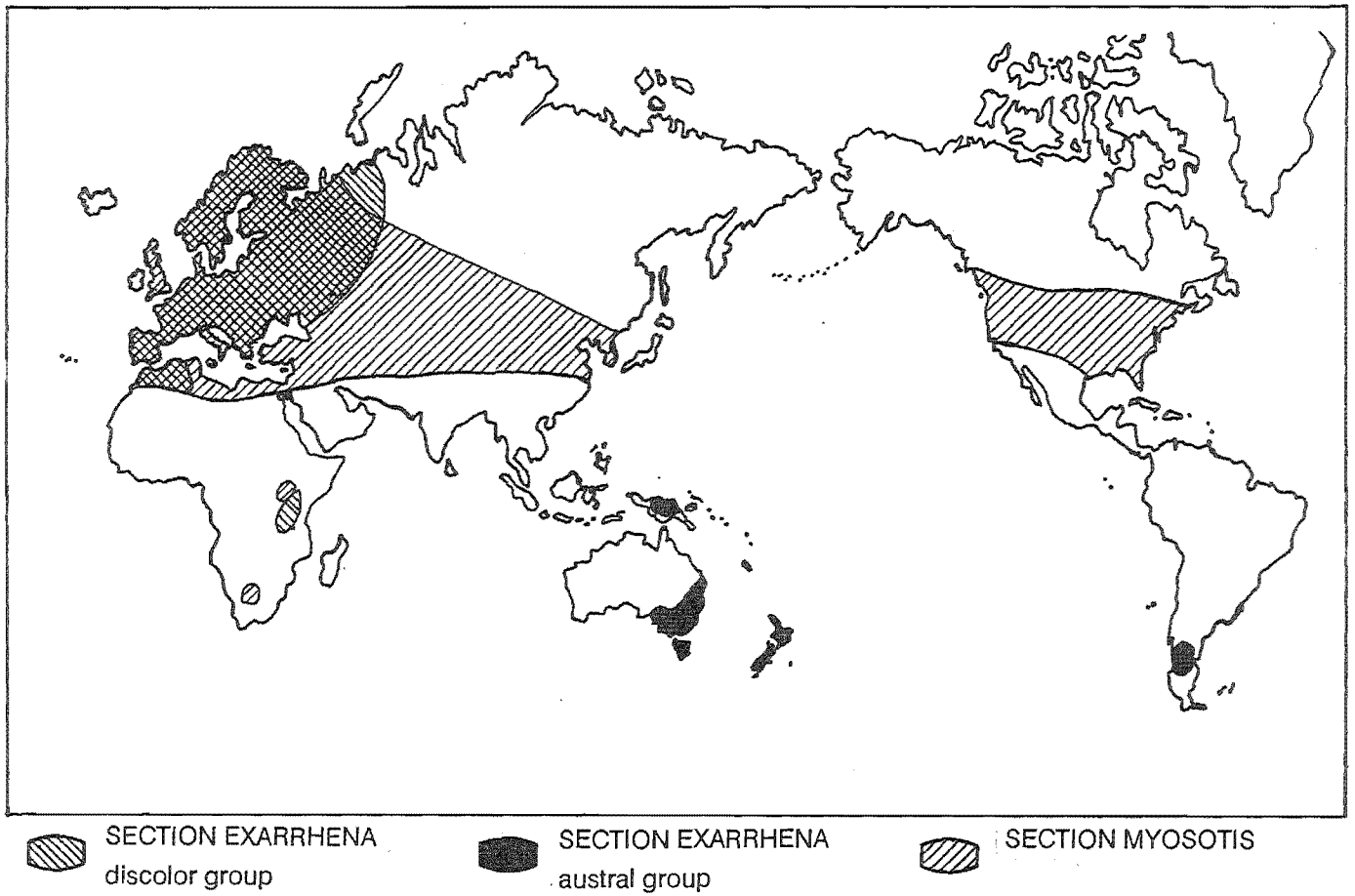


Figure 1. The distribution of the main groups of *Myosotis*.

SYNOPSIS OF THE GENUS *MYOSOTIS*

(A) Section Myosotis

All Eurasian species, with the exception of the *discolor* group, including the North American *M.verna* and the South African *M.vestergrenii* and *M.semiamplexicaulis*.

(B) Section Exarrhena

(I) *Discolor* group.

(a) Europe - *M.discolor s.l.*; *M.persoonii*; *M.balbisiana*; *M.congesta*

(b) East Africa - *M.abyssinica*

(II) Austral group

(a) bracteate lateral inflorescence

M."non-pulvinaris"; *M.pulvinaris*; *M.cheesmanii*; *M.glabrescens*; *M.uniflora*; *M.colensoi*; *M.lyallii*; M."pymaea"; M."drucei"; M."minutiflora"; M."glauca"; M."volcanic plateau"; *M.antarctica* (sub-antarctic islands); *M.albiflora* (South America); *M.spathulata*; *M.tenericaulis*

(b) ebracteate erect inflorescences

(i) lowland forest group - *M.forsteri*; M."petiolata"; M."pottsiana"; M."pansa"

(ii) *australis* group - *M.australis* (Australia); M."yellow"; M."sm white"; M."calc white"; *M.saruwagedica* (New Guinea)

(iii) *goyenii* group - *M.goyenii*; M."lyttletonensis"; M."poolburn"; M."Mt somers"; *M.albosericea*

(iv) *rakiura* group - *M.rakiura*; *M.capitata* (Sub-antarctic islands)

(v) *angustata* group - *M.angustata*; *M.eximia*; *M.arnoldii*; *M.laingii*; *M.saxosa*; M."livingstone"; M."takitimu"

(vi) *monroi* group - *M.monroi*; *M.laeta*

(vii) *traversii* group *M.traversii s.l.*

(viii) *brockiei* group - *M.brockiei*; *M.concinna*

(ix) uncertain affinities - *M.explanata*; M."fiordland"; *M.exarrhena* (Australia); *M.macrantha*; *M.oreophila*

Species regarded as synonyms include M.*venosa* (syn. of *M.forsteri*); *M.suavis* (syn. of *M.explanata*); *M.elderi* (syn. of *M.lyallii*); *M.amabilis* (syn. of *M.saxosa*); *M.matthewsii* (syn. of *M.spathulata*).

Biogeography

A discussion of the biogeography of any southern group is not complete without the mention of Joseph Hooker. His introductory remarks to the Flora Novae-zelandiae (Hooker, 1853) provided a remarkable insight into the historical biogeography of a region whose importance is such that it continues to be argued about today. When confronted with the strong similarities in the floras of different parts of the austral region, Hooker concluded that such patterns could not be the result of long-distance dispersal, but must be the caused by the breakup of some ancient formerly continuous land-mass. As such, he might be regarded as the father of vicariance biogeography. It was more than a century later that a mechanism to account for Hooker's observations became generally accepted. The theory of continental drift and plate tectonics has led to one reviewer to conclude that:

> "....It seems clear today that many of the views and conclusions of Darwin and Wallace and their followers have to be laid to rest, and that Hooker (1853) was right in principle.... " - Brundin (1988).

Brundin's mention of Wallace and Darwin are testimony to the strength and merit in some cases of the opposition to Hooker's claims. Wallace (1876) and Darwin (1859) were both working under the assumption that the continents had been stable over time. They also assumed that the center of evolution for most groups was in the north and that the southern lands acquired organisms from the north by long-distance dispersal. Brundin is premature in his assertion that these ideas of Darwin and Wallace have been lain to rest. Long-distance dispersal from areas to the north still feature strongly in the writings of recent workers on plant biogeography, particularly with regard the evolution of the alpine group of plants of Australia and New Zealand. Raven (1973) argued very strongly that the bulk of New Zealand's alpine flora is an assemblage of cosmopolitan taxa that arrived by long-distance dispersal via Indo-Malaya and through Australia to colonise the newly formed habitats that were created as mountains were uplifted in the Miocene and Pliocene. *Myosotis* is included in the list of taxa that Raven's lists as arriving in New Zealand in this manner. This is spite of the acknowledgement of Grau's finding that it is the northern group of species that are relatively derived rather than the other way around. Raven's chief objection to an older southern origin for this group seems to be the relative age of the sympetalous orders of angiosperms. He asserts that old vicariant events could not explain the

distribution of groups like *Myosotis* as they were "simply not around early enough". Raven's viewpoint has become the current orthodoxy concerning the biogeography of these groups (eg Mildenhall (1980)). Wardle (1968, 1978), although apparently concurring with the age of the evolution of the Sympetalae, has argued that a proportion of New Zealand's alpines may have evolved *in situ* from lowland relatives. Moreover, in some cases, where distributions occur within New Zealand and Australia, he argues that long-distance dispersal has occurred in the other direction - from New Zealand to Australia. I will suggest here that the distribution of *Myosotis* is possibly best understood as being the result of a southern origin at a time when connections between Australia, South America, New Zealand, and possibly Africa still existed. On this view, the subsequent disjunction of the genus is the result of vicariant and extinction events at a later date.

The work of Grau and co-workers strongly suggests that the sister group of the southern species is the *discolor* group. This group is found in eastern Europe centered in the southern Mediterranean but with an outpost in the highlands of East Africa. The Eurasian section Myosotis, is relatively uniform and derived in its pollen and other features. It would seem possible therefore, that the route of migration was north through Africa and not south through Asia and New Guinea as postulated by Raven and others. Raven (1979) provides a useful summary of the important tectonic events with respect to biogeography. The Australian plate (including New Zealand and New Guinea) was widely separate from Asia and Malaya until the end of the Eocene. South America, New Zealand, Australia and Africa on the other hand, were in close contact until the late cretaceous when firstly Africa, followed by New Zealand, Australia and finally South America separated from the Antarctic continent. Africa and Europe were in contact for much of this period, but North and South America did not meet until the Eocene. Thus we see that a group originating in Gondwanaland in the late Cretaceous would have migratory routes across land into all the areas that *Myosotis* now occupies and that the split of the continents matches in part the split that is seen in the basic groupings of the genus. One might expect however, that because Africa split off first from the rest of Gondwanaland and the other parts later, the primary split in the genus should be between the African/Eurasian species and

the austral group. Grau and co-workers, however, prefer a primary split of Eurasian and Southern species plus the discolor group. Such an anomaly could arise in two ways. It could occur as a result of recognising plesiomorphic states as characters that unite the southern and discolor species together (which can only be resolved by assigning polarity to the characters concerned). Alternatively, it may have been the result of a prior differentiation of the Eurasian species before the separation of Africa from Gondwana. The alternative route between the hemispheres through island chains in Asia is not supported by the current distribution or taxonomy. Dispersal south through such a route is not consistent with Grau's contention that the Eurasian species are derived or the intermediate position of the East European and African species. It is clear that the best hope of revealing the true pattern must lie with a thorough cladistic investigation involving all three major groups of species.

Myosotis does not appear well suited for long distance dispersal. The seeds are dry and not attractive to birds and there is no special mechanism to promote dispersal ability. A northern origin and subsequent dispersal would require jump dispersal events across Europe, through Asia, across the Pacific, and the Tasman and to the sub-antarctic islands. If such dispersal has occurred recently, it has done so without leaving any intermediates over much of this route.

The presence of *Myosotis* in *Nothofagus* forests of New Guinea may be regarded as evidence of an ancient association. These forests are the habitat of many of the taxa that are accepted to be gondwanic in age. The absence of *Myosotis* from New Caledonia, which has been regarded as a museum of Gondwana relicts (Raven, 1979) is problematical, but may represent a failure to adjust to climate changes as the paleolatitude of New Caledonia drifted north.

The main problem however, with supposing that the distribution of *Myosotis* precedes the breakup of Gondwana, and many would regard it as a problem large enough to reject the hypothesis outright, is the lack of a fossil record beyond the tertiary. The oldest record of pollen of the family is from the Oligocene in Puerto Rico (Muller 1981). From New Zealand, no pollen

has been found beyond the Quaternary (Mildenhall 1980). However, as many others have pointed out (eg Brundin 1988), the oldest fossil simply establishes the minimum age of a group. Where a group is seldomly encountered in pollen records, the gap between the oldest fossil and the age of a group may be considerable, based on the low probability of fossilisation. A rare, non-bog, insect-pollinated group is not a good candidate for preservation in the micro-fossil record as an inspection of pollen cores will show. For instance, P. Randall (pers comm) has shown that of the pollen encountered in the modern pollen rain of a transect through Canterbury and the main divide, less than 5 % of pollen is from animal-pollinated plants and less than 0.5% appears to be from insect-pollinated herbs that do not grow directly on the bogs he was sampling. Some very important groups such as the Scrophulariaceae were represented by only a handful of grains from the many tens of thousands of grains identified.

Microfossil records of a genus like *Myosotis* are likely to be very few. However a significant macrofossil record of the Boraginaceae has been discovered from the Kamchatka province of the U.S.S.R. (Chelebajeva 1984). Chelebajeva claims to have discovered the Boraginaceous tree *Cordia* from Palaeogene sediments. He also suggests that the type genus *Grewiopsis* may also be assigned to *Cordia* and that *Cordia* "was widely distributed throughout the northern hemisphere from Late Cretaceous till Early Oligocene." If this finding is vindicated it will be of major significance to ideas on the history of the family. It would make it at least possible that *Myosotis* has an equally long history in the southern hemisphere.

The distribution of certain groups of species of *Myosotis* within New Zealand also supports a relatively old age for the genus in New Zealand. Figure 2 shows the distribution of the advanced *angustata* group of species. This group is characterised by an advanced pollen type that is not found elsewhere in the genus. Each species occupy an extremely restricted range. Each species is found on a single mountain or a small group of mountains. Where they do occur, however, they are relatively abundant. Two large disjunctions are encountered in the distribution of this group. Three concentrations of species occur, one in the central region of the North Island, one in North-West Nelson and the third in Western Otago/Fiordland.

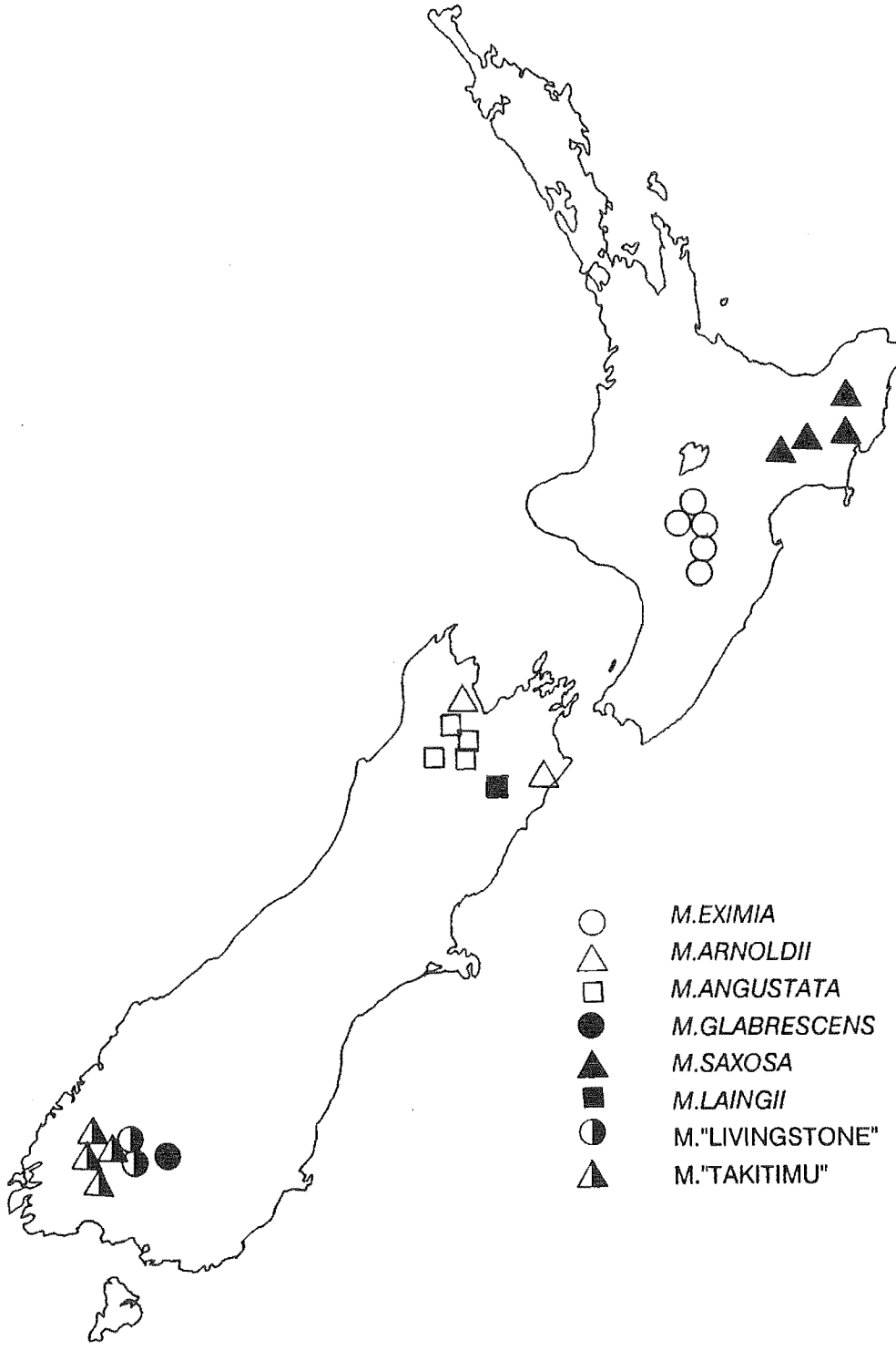


Figure 2. The distribution of the angustata group of species. The symbols represent all known populations of each species.

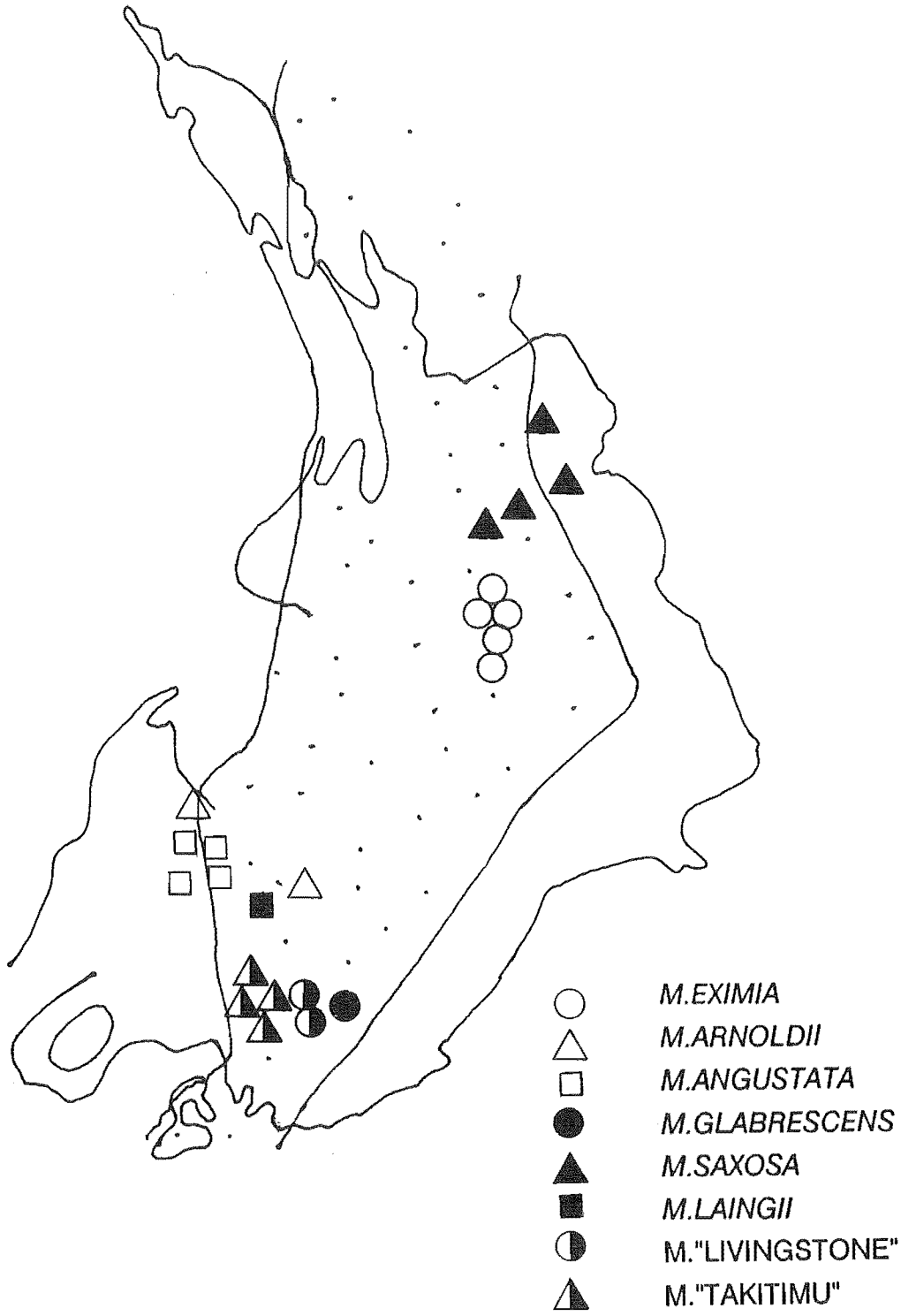


Figure 3. The distribution of the angustata group of species superimposed on a reconstruction of New Zealand in the late Oligocene (after McGlone 1985).

Distribution patterns such as this are common for many groups of angiosperms (Wardle 1963, Burrows 1965, Druce 1984, McGlone 1985). Traditionally, such disjunctions are explained as the result of past episodes of glaciation. However, recently, a new explanation involving tectonic events in the Cenozoic has been suggested (McGlone 1985). McGlone argues that two major events - the trans-dextral displacement of the south island along the alpine fault, and the occurrence of an extensive sea strait across the lower half of the North Island, are responsible for the common patterns of disjunction evident today. Figure 3 shows a palaeographic map of the late Oligocene from McGlone (1985). On it are plotted the distribution of the taxa of the *angustata* group in the areas they occupy today. This map reveals that one of the disjunctions - between Otago and Nelson is no longer evident. The other disjunction between Nelson and the central North Island is presumably younger and the result of the Pliocene sea-strait in central New Zealand.

Other similar disjunctions also occur in *Myosotis*. In particular, *M.tenericaulis* (one population in central North Island, with a wider distribution in Nelson/Marlborough and Otago/Southland); and "M.glauca" (Central Otago and central North Island).

If the above scenario of vicariance is correct, *Myosotis* must have been in New Zealand prior to the Miocene. If this is true of *Myosotis* then it presumably could be true for other members of the Sympetalae. Barker (1982, 1986) has recently argued that *Euphrasia* (Scrophulariaceae) may also have had a similar history. The possible find of pollen attributable to the family from the late Cretaceous (Mildenhall 1980; Barker (pers comm)) may be important evidence, if it is confirmed.

In summary, an argument that *Myosotis* appeared in New Zealand (and other austral parts of gondwana) somewhere near the late cretaceous boundary is presented. Migration northwards may have occurred via Africa to Europe subsequently. Vicariant events and habitat changes within New Zealand locally and more extensively may have prompted speciation since the Oligocene. In particular, the upthrust of mountain habitats in the Miocene presented

opportunities for radiation into new habitats. Such a pattern of evolution is consistent with current concepts of taxonomy of the genus. Without a good phylogeny, however the biogeographic history remains uncertain. The application of cladistic techniques using sound characters may throw light on which of the conflicting alternatives best explains the pattern of evolution of *Myosotis*.

DESCRIPTIVE FLORAL BIOLOGY

In this section, the general flowering features of *Myosotis* are described.

The inflorescences are indeterminate cymes borne on lateral branches arising from the base of the central rosette. The flowering stems may be simple or one- or two-times branched. In some species, the flowering stems are erect and ebracteate with short stem leaves in the non-flowering part of the stem. In other species, the inflorescences are prostrate, with a leafy bract subtending each of the flowers.

The 5-merous flowers are usually tubular and are made up of five petals which are fused to about half-way forming a tube surrounded by a wide rim of free petal lobes. At the point of divergence of the petals and the tube, there are a series of folds of the corolla to form "scales". The anthers are epipetalous and borne on filaments of varying length. Pollen is shed by the longitudinal split of each of the two anther sacs. The stigma is held aloft on a style of variable length, and may be variably two-lobed. The ovary is deeply four-lobed and each of the uniovulate lobes matures into a shiny nutlet. These are attached near their base to the receptacle. The gynobasic style emerges from between the lobes of the ovary. Near this point are the nectaries which are indistinct and partly enclosed by another enfolding of the corolla tube similar to scales described above. The calyx is deeply five-lobed and persistent and encloses the nutlets as they mature. There are no special mechanisms of dispersal of the nutlets - they simply are shaken out by the wind or fall to the ground when the flowering stem abscises. Most species are short-lived perennials but some species may be annual or ephemeral (eg M."minutiflora").

Figure 4. Dichogamy in *M.colensoi*.

a. Just after opening the flowers are in an exclusively female phase.

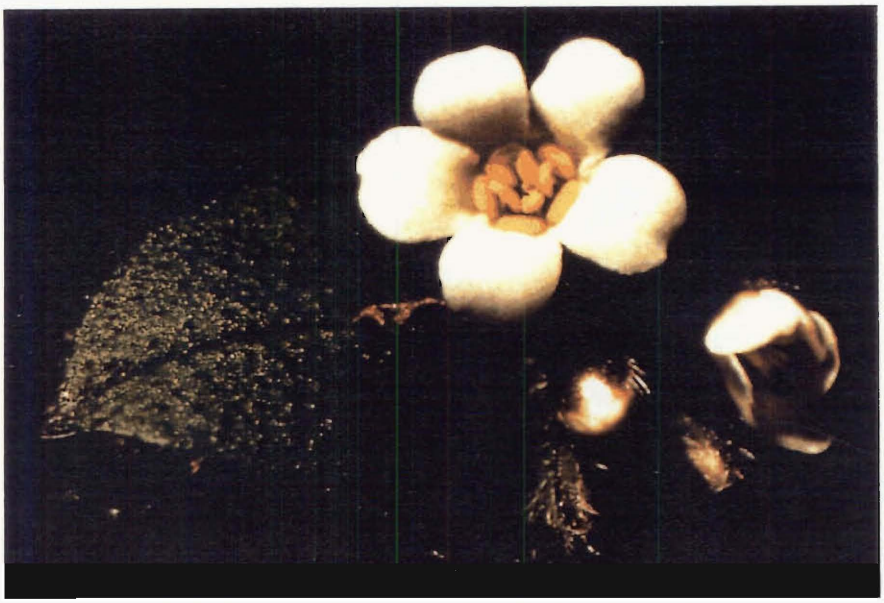
b. The anthers begin to dehisce.

c. After at least a day, the corolla scales fade, pollen is no longer presented and the stigma is brown and dry.

The flowers are protogynous. There is a short delay after flowers open before the anthers dehisce. The stigma, however, is accessible and receptive as soon as the flower opens. In some cases, the stigma is able to be naturally pollinated *before* the flower opens (see the description of brush blossoms below). Nectar is the usual reward for pollinators, however. After a variable amount of time the anthers dehisce. Pollen is presented directly on the surface of the anthers. Some time after the anthers have dehisced, there is usually a period when pollen is not available and stigmas are unable to be successfully pollinated. This change to a post-presentation phase is usually signalled by a colour change to the whole flower (some populations of *M.uniflora*) or to the corolla tube and scales, or to the scales alone. After this change, there is no apparent direct function either in terms of pollen donation or pollen receipt. The role of such flowers may best be understood as mechanisms to promote the rate of visitation to the remaining flowers (see Chapter 4). As an example of the typical sequence of floral dichogamy, Figure 4 shows the three main phases of *M.colensoi*. The female phase (Figure 4(a)) is short and is quickly followed by the male phase (Figure 4(b)). This phase lasts longer than the female phase and after approximately two days the corolla scales fade and the flower enters the post-presentation phase (Figure 4(c)). Pollinators do not usually probe flowers that are in this phase (Chapter 3). For more details of the floral sequence in *M.colensoi* see Chapters 2 and 3.

Floral syndromes

Myosotis shows a wide variety of floral forms in New Zealand. This diversity of form is matched by an equally diverse pollinating fauna. Despite the wide variety of form, it is possible to recognise within the New Zealand species common patterns or syndromes in the sense of Faegri and van der Pijl (1971). Three principle types are recognised, although of course variation exists within these types and some species are difficult to place firmly in only one class. Each class will be described and two examples shown. An attempt is made to assign each species to a syndrome.



Tube Flowers

Figure 5 (top). A flower of *M.forsteri* from Mt Arthur in female phase.

Figure 6 (bottom). *M.traversii var traversii* from Rainbow ski-field.

(1) Tube Flowers

The majority of species exhibit this syndrome. The flowers resemble in shape the flowers of the common forget-me-not of gardens *M.sylvatica*. The flowers have a narrow corolla tube of varying length, which is usually rimmed by well-developed corolla scales. The corolla lobes are free and open out flat to form a wide brim. The tube is narrow and usually precludes pollinators from directly entering the tube (but see the description of pollination of *M.uniflora* by ants below). Therefore, in order for pollinators to reach the concealed nectar, they must have a proboscis long enough to reach the bottom of the tube. The anthers are located near the top of the tube, although their exact position varies. Some species have anthers that are more or less exserted beyond the tube, while in other species the anthers are contained well within the tube. Moreover, some species show quite a lot of variation between different localities in their exact degree of anther exsertion. For instance, the population of *M.forsteri* shown in Figure 5 has anthers that are partly exserted beyond the floral tube. In other populations of *M.forsteri*, however, the anthers are contained entirely within the tube. The stigma also varies in its position and may be above the anthers and beyond the tube, or contained within the tube and below the anthers. This variation of anther and stigma position within the tube-flower group, coupled with the differential growth of the corolla tube and style throughout the life of a flower, allows variation in the timing and likelihood of autogamous selfing. The complete range from pollinator-requiring forms through to autonomous selfers are encountered within this group. Between species and plant variation in herkogamy is considered in more detail in Chapter 2. The other representative of the tube-flower group depicted (Figure 6) is *M traversii var traversii*, in which the stigma begins above the anthers but is overtaken during the life of the flower and finishes beneath them.

Other species that can be assigned to the tube-flower syndrome include: M."non-pulvinaris", *M.pulvinaris*, *M.colensoi* , *M.cheesmanii*, *M.glabrescens*, *M.lyallii*, M."pymaea", M."drucei", M."minutiflora", M."glauca", M."volcanic plateau", *M.antarctica*, *M.albiflora*, *M.spathulata*, *M.tenericaulis*, *M.australis*, M."yellow", M."sm white", M."calc white", *M.rakiura*, *M.capitata*, M."livingstone", M."takitimu", *M.explanata*, M."fiordland", *M.oreophila*.

Funnel Flowers

Figure 7 (top). *M.goyenii* from the Broken River population.

Figure 8 (bottom). *M.macrantha* from the Mount Cook population.

(2) Funnel Blossoms

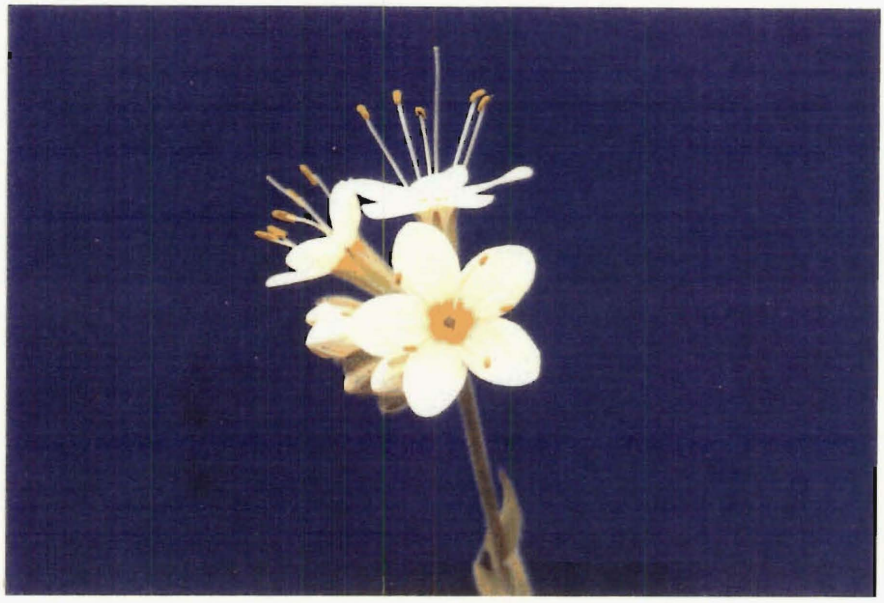
A second syndrome of floral form may be conveniently called the funnel-blossom. In these species, the corolla lobes never completely flatten but flare outwards in a or funnel- shape. *M. goyenii* is an example of this type (Figure 7). The tube is wider than in the tube flowers so access to the nectar is possible by partly crawling into the tube. The funnel-shape nature of the flower is further developed by a partial fusion of the corolla lobes above the scales. The scales therefore take an inferior position and are usually less evident than in the previous type.

M.macrantha (Figure 8) and *M.albosericea* (Figure 1a) are further examples of this type. Other representatives include: M."lyttletonensis", "M.poolburn", *M.arnoldii*.

(3) Brush Blossoms.

A third group of relatively restricted species, centered in North-West Nelson and the central North Island, are the brush blossom species. Two examples, *M.brockiei* and *M.monroi* are illustrated in Figures 9 and 10. These species are characterised by bearing anthers and stigmas on long exserted filaments or styles. Beneath these is a usually narrow corolla tube rimmed by conspicuous corolla scales. In some cases, e.g. *M.eximia*, the tube is relatively open and funnel-like but the main feature of having exserted floral parts is retained. No species of this syndrome has been studied at flowering in natural conditions. Pollinators presumably must contact the anthers and stigma in a rather haphazard way and presumably become covered in pollen.

A feature of the brush blossoms is the relative synchrony of flowers in an inflorescence. Complete synchrony is not possible as the inflorescence is indeterminate and flowers must develop sequentially. However more synchrony is achieved in these species than in either of the previous groups. This occurs because of three floral features. Firstly, the stigma presentation in the buds is precocious. That is, the stigma is commonly exserted beyond the enclosed petal lobes (Figure 11).



Brush Blossoms

Figure 9 (top). *M. brockiei* from the Arthur Tableland.

Figure 10 (bottom) *M.monroi* from Dun Mt., Nelson.

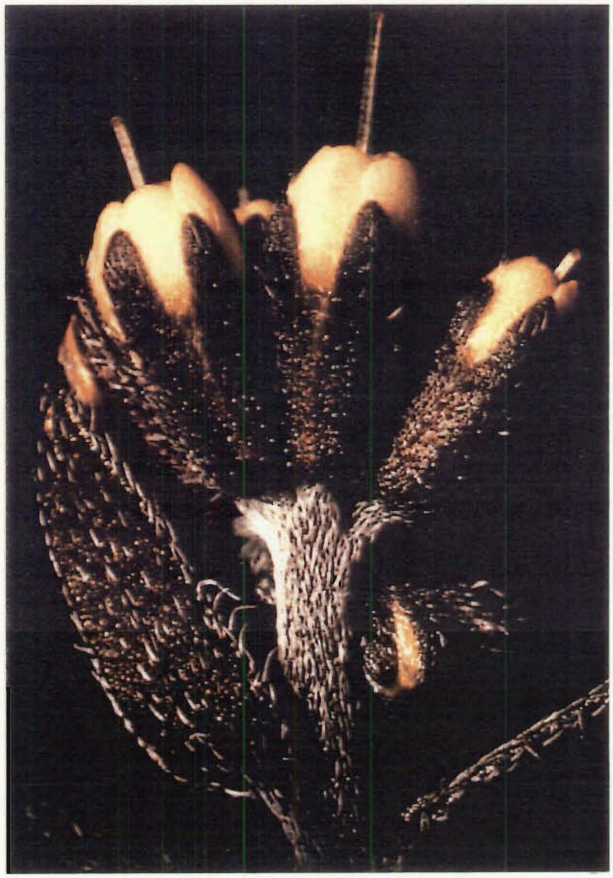


Figure 11. *M.angustata* from Mt. Arthur. Note the precocious buds.

This has the effect of presenting the stigma for pollination before the flower opens and possibly while the older flowers are still in the female phase. Secondly, the time over which the different flowers of an inflorescence open is reduced, and the individual flowers are closer together in their developmental stage. Finally, the relative length of the female phase is greater than in most other species. The combined result of the three features is that the majority of flowers have at least some opportunity of pollination before the anthers begin to dehisce.

Other species with this type of flower include the following: M."petiolata", M."pottsiana", M."pansa", *M.angustata*, *M.laingii*, *M.laeta*, *M.saxosa*, *M.concinna*.

Table 1. The distribution of floral syndromes in the taxonomic groupings proposed above. Representative taxa are indicated.

Taxonomic Group	Flower Syndromes	Representative Taxa
Section Myosotis	All tube flowers	*M.sylvatica*
Section Exarrhena		
discolor Group	All tube flowers	*M.discolor*
Austral Group		
Bracteate Group	All tube flowers	*M.uniflora*
		M.colensoi
		M.spathulata
		M."minutiflora"
Ebracteate Group		
Forest Group	Some tubes	*M.forsteri*
	Some brushes	M."petiolata"
goyenii Group	All funnels	*M.albosericea*
		M."lyttletonensis"
		M.goyenii
rakiura Group	All tubes	*M.rakiura*
angustata Group	Some tubes	*M.glabrescens*
	Some funnels	*M.arnoldii*
	Some brushes	*M.eximia*
monroi Group	All brushes	*M.monroi*
traversii Group	All tubes	*M.traversii var traversii*
brockiei group	All brushes	*M.brockiei*

POLLINATION AND DESCRIPTIONS OF SELECTED SPECIES

During the course of the study, the pollination biology of several species was examined. It had been intended to make a general survey of all species and their pollinators. However, it became apparent that such a task was beyond the scope of the thesis. Difficulties in visiting isolated species with short flowering periods and shy pollinators made it clear that this type of data will take many field seasons to collect. However, some information on pollination and general floral biology was collated for a handful of species. This section outlines some of this work. The section also acts as a introduction to the species *M.colensoi* which is considered in

more detail in the following chapters.

Flower visitors were collected when observed and later identified as far as possible. P.N. Johns of the Zoology Department of the University of Canterbury made all identifications except the noctuid moth which was identified by Graham White. Vouchers are deposited in the Zoology Department of the University of Canterbury.

M.colensoi

This species was previously thought to be restricted to tertiary limestone in the Broken River Basin in Canterbury (Moore,LB 1961). However, Druce and Williams (1989) report that the species has been found on a similar substrate in Marlborough. Thus the species may have once had a larger distribution and has recently become restricted to the increasingly rare tertiary limestone remnants. Such marine sediments would have been common before the extensive mountain building process began in the Miocene. The possibility of more recent dispersal cannot be eliminated, however.

I have not seen material from Marlborough and all observations of the floral biology of this species were made at Castle Hill Station (grid reference NZMS 1 S66 194 943). At Castle Hill, the species is commonly found on limestone rubble in full sunlight and on favourable sites on the limestone tors. In good positions, densities of plants can be quite high and often is a major component of the ground cover.

The plant is a prostrate perennial and has the relatively unusual habit for *Myosotis* of forming fairly large patches, up to about 0.4 meter diameter. It achieves this a prostrate habit by lateral spread from the central rosettes. New lateral rosettes are formed from the bases of the old rosettes which spread out across the ground. This process continues slowly until an old plant consists of perhaps a dozen interconnected rosettes.

Flowers appear at Castle Hill in the last week of October and plants continue to flower

until mid-December. Individuals have a shorter flowering period than this, however; each plant produces new flowers for about a month. There is a progression of flowering starting with plants on the most northerly aspects, while plants on the less sunny southern aspects begin and end their flowering periods later. Large plants produce many flowers simultaneously over the entire patch, each branch of each rosette opening and maturing flowers successively. The result is a large display of flowers at all different stages, asynchronous in their dichogamy.

The pure white flowers are relatively large (Figure 4) with a tube of about 7 mm (see Chapter 2). The corolla scales are well developed and bright yellow in the presentation phase. The anthers are contained entirely within the floral tube though the tips extend to the level of the corolla scales. The stigma is well exserted beyond the floral tube and remains so throughout the life of the flower (see Chapters 2 and 3 for details of the herkogamy and dichogamy of this species).

The most important flower visitor is a large tachinid fly *Protohystricia huttoni* (Malloch). This fly is a specialised nectar feeder as an adult and has been found previously on *Isotoma fluviatilis, Pratia angulata, Celmisia gracilenta* and *Cirsium arvense* (Primack 1983). It has a long proboscis (about 7mm) which it uses to probe nectar. The fly is active on *M.colensoi* for most of the middle part of dry and warm days. It begins foraging at approximately 10 am and continues throughout the day until about 5 pm. In the morning, much of the time is spent making exploratory flights to plants, often visiting one flower only before flying rapidly off to another. These flights appear to be gauging when flowers begin to produce nectar, although the timing of nectar production was not confirmed. Later as temperatures rise, feeding trips begin. Then, flights between plants are typically slower and over shorter distances than the earlier exploratory flights. Once on a plant during a feeding flight, many flowers are successively visited as the fly crawls over a patch. Not all flowers encountered are probed and post-presentation flowers particularly are often approached but left without being probed (see Chapter 3). When a flower is probed, the proboscis is inserted into the floral tube to collect nectar from the base. In doing so, the insect's head comes into contact with the anthers. Flies have been observed with large

accumulations of pollen around the front of the head. The stigma is contacted first as the fly crawls over the flower; it does not require insertion in the tube. *P.huttoni* was not observed to forage on other flowers except occasional apparently mistaken visits to the common adjacent flowers of *Stellaria*. This mistake is usually realised immediately and foraging on *M.colensoi* quickly resumes.

Other visitors were also observed on *M.colensoi*. Perhaps the most important was another unidentified tachinid which, although primarily concerned with collecting nectar from *Stellaria* flowers, were sometimes observed to make long foraging bouts on *M.colensoi*. It was much less abundant and less persistent than *P.huttoni* but it may cause a small amount of pollination. A small native solitary bee (unidentified) also visited *M.colensoi*. It usually appeared around midday on only the warmest days. It is not big enough to regularly contact the stigma, which is exserted too far for the body of the rather precisely flying bee to contact. The bee seems to collect only pollen. It may therefore have a significant effect on male fitness by robbing pollen, but its importance in affecting successful pollinations is probably minor. Other visitors were much less frequent and included one honeybee, and very occasional visits from a syrphid *Melangyna novae-zelandiae* (MacQuart) and the introduced calliphorid *Chysomyia rufifacies*. *M.colensoi* thus seems to be chiefly pollinated by one main visitor, *P.huttoni*. Other visitors are much less important, although the effect of the native bee on pollen removal may be significant.

M.colensoi is self-compatible. Table 2 shows the result of hand-pollinations in the field on plants enclosed in mesh to prevent the access of pollinators. Several plants were chosen and covered with mesh enclosures. After about one week, all flowers on the plant had opened while under the mesh and were therefore unpollinated. Flowers were chosen at random and checked with a 20x hand-lens to ensure that no pollen had been deposited on the stigma. The flowers were then hand-pollinated with either of two types of pollen. Half the flowers were pollinated with pollen from flowers from the same plant (in some cases from the flower's own anthers). In 1987, pollen from one other plant at least 5 metres away was used for the cross-pollinations. In 1988 mixtures of two separated plants were used for the cross-pollinations and the pollinations.

Table 2. The results of self- and cross-pollinations for the 1987 and 1988 seasons.

Treatment	Mean Seed set (max. 4)	n	Standard Deviation
1987 Season			
Self-Pollination	3.07	68	1.36
Cross-Pollination	2.97	32	1.64
1988 Season			
self-pollination	1.04	75	1.62
cross-pollination	0.80	148	1.51

ANOVA - 1987 Data

Source of variation	df	S.S.	F	P
Model	11	26.0	1.14	0.3378
Plant	5	21.9	2.12	0.0703
Treatment	1	0.01	0.03	0.8658
Interaction	5	2.03	0.20	0.9632
Error	88	181.8		

1988 Data

Source of variation	df	S.S.	F	P
Model	31	238.8	5.04	0.0001
Plant	15	194.4	8.47	0.001
treatment	1	3.18	1.80	0.1991
Interaction	15	26.4	1.15	0.3126
Error	191	292.1		

These are mixed model anovas, so the treatment mean squares were tested over the interaction mean squares. Type III sums of squares were used to evaluate the F-ratios.

No significant differences in seed set per fruit was found in self- and cross-pollinated plants. Data for two seasons is presented, and though the overall mean for the 1988 season is lower than that of the 1987 season, the decrease was similar for both types of pollination. It appears that the 1988 season was for some reason a poor year for fruiting for *M.colensoi*. Not only was seed set down on the previous year, but studies of seed germination indicated that the average viability of the seeds was also lower (unpublished results). The reason for the difference between the seasons is unknown but may it be the result of a noticeable increase in the powdery mildew *Peranospora myosotodis*, which apparently occurs naturally on this and other species of *Myosotis* (Cartman 1985).

M.macrantha

This species occurs throughout the length of the South Island on the main mountain ranges, although it is more common on the eastern side of the divide. It characteristically inhabits steep south-facing rocky bluffs, usually above 1200 M. Populations are usually small and plants are scattered and difficult to study. At one locality in Mount Cook National Park (grid ref NZMS1 S79 768 375), however, a very large population exists where very many plants occur together on a gently sloping gravel bank. The population is thus an ideal subject for study. Several trips were made to the populations during the summers of 1986-1987, 1987-1988 and 1988-1989.

This species is probably a relatively short-lived perennial which flowers annually over the course of several seasons. Flowering begins in late December. The large rosette leaves (up to 150 mm) produce one to several erect flowering stems that can reach 400 mm in length. Flowers are produced for several weeks sequentially on each inflorescence as the cyme uncurls. There are usually about 6 flowers open on an inflorescence at one time and there may be several branches on a stem, each bearing a cluster of flowers, and up to 6 stems per plant. Thus there is usually a large display of flowers which lasts for several weeks.

The funnel-shaped flowers are large with a long tube approaching 15mm and wide flaring

lobes (Figure 8). The scales are inconspicuous and well down the mouth of the tube. The style is very long and in some cases may protrude from the buds before the flower opens (see the description above of "brush-blossoms"). The anthers are contained within the tube and are widely separated from the stigma. The species displays a remarkable range of flower colours. Each plant is consistent in its flower colour, but adjacent plants within a population show widely differing colours. The flowers range from a rich chocolate purple through bright purple, yellow purple, bright yellow, blue-yellow and a pale lemon. It is generally possible to assign individuals to discrete flower cases though in some cases classification is difficult. Several components of colour appear to control the hue of a particular plant. The components appear alone or in combination with others to produce a wide array of phenotypes. Nothing is known of the genetics of the colour polymorphism but several genes are apparently involved. No other species of *Myosotis* has found that approaches the range of flower colours shown in this species but some species (eg. *M.arnoldii*) have dark brown or purple flowers and others species are yellow.

Despite the large showy and coloured flowers, *M.macrantha* is not visited during the day. Many hours of observations in the first two flowering seasons during the day resulted in only a single visit from a bumble bee. During the 1988-1989 flowering season, however, a trip specifically to look for night-pollination revealed that at dusk, the flowers are visited by the noctuid moth *Aletia cuneata*. This large moth was observed to be actively probing flowers of *M.macrantha* just as dark was falling. This observation, coupled with the lack of flower visitors during the day and the observation that pollen was found to be regularly deposited on flowers overnight and not during the day, leads me to believe that pollination is carried out only at night by *Aletia cuneata*. It would be of interest to know whether nectar production coincides with dusk and whether *Aletia cuneata* also visits *M.macrantha* in other localities.

M.uniflora

This species occurs in the South Island from Central Otago to the Waimakariri River, on large stable riverbeds in the rain shadow east of the main divide. The population studied was on the Tasman river bed near the outlet of the Tasman Glacier (grid ref NZMS1 S79 810 329). The species is very specific in the age since disturbance of the surface it inhabits. It apparently requires a stable substrate for colonisation but is eventually out competed by other native species and weeds on older surfaces. The patches of suitable habitat are often small and are always in danger of being washed away. The plant forms mats of creeping stems and is unusual among *Myosotis* in not forming definite rosettes. The leaves are also unusually small and narrow and the convergence of the habit with other mat-forming genera such as *Raoulia* is striking. Flowering occurs in late November and is completed by mid-December. The flowers are produced as small laterals and usually only one flower per branch is produced. Many flowers may be open on a plant at one time. The flowers (Figure 12) are tubular and in some populations are unusual in that the complete flower undergoes a colour change as they enter the post-presentation phase (details of the herkogamy and dichogamy of this species are given in Chapter 2).

After many hours of watching during the day, the only visitor to the flowers observed was the native ant *Tetramorium antarcticum* (White). Individuals were commonly observed crawling into the floral tube to collect nectar from the base (Figure 12). In doing so, an ant's body became covered in pollen which adhered to the short body hairs. Pollination was regularly observed as the body contacted the exserted stigmas. Ants only infrequently leave the plant they are on as they appear to live in nests amongst the plants of *M.uniflora*. Thus there appears to be little opportunity for cross-pollination when ants visit the flowers. It would appear that *M.uniflora* may not be adapted for ant-pollination as the almost inevitable geitonogamy will result in very high levels of self-pollination. The cheaper alternative of autogamous self-pollination would presumably be selected under these conditions. It is possible that other visitors visit the flowers, perhaps at night. Nevertheless, it is clear that ants functionally will achieve many pollinations, albeit self-pollinations.

Figure 12 (top). Native ants on the flowers *M.uniflora*, Mount Cook.
Figure 13 (bottom). *M.albosericea*, Dunstan Range. Note the small beetle in one of the flowers.

M.goyenii

This species occurs in scattered localities east of the divide from Central Otago to North Canterbury where it inhabits some of the very driest areas of the South Island. It usually occurs on more southerly aspects of steep rubbly cliffs on ledges and in crevices. The population studied was at the confluence of Sloven's creek and Broken River in the Waimakariri catchment (grid ref NZMS1 S66 356 977). Here it is found on an outcrop of semi-schistose tertiary sediments which are rich in mica.

The habit is much like that of *M.macrantha*. However, the rosette leaves are smaller and the inflorescences are laxer and more sprawling. A large plant has many stems which bear clusters of several flowers. Flowering begins in October and lasts for approximately six weeks. The flowers are white with pale cream-yellow corolla tubes (Figure 7). The flowers are funnel-shaped and the tube extends beyond the corolla scales as the flower lobes are partly fused beyond this point (see above). The stigma is well exserted beyond the relatively small corolla scales that are positioned well down the extended corolla tube. The style is often slightly off-center with the distal portion bending over slightly to bring the stigma into a more central position.

The funnel-shaped flowers are apparently visited exclusively by the fly *Helle longirostris* (Hudson), a member of the family Acroceridae. This fly has also been recorded on the flowers of *Celmisia discolor* and *C.spectabilis* (Primack 1983). Only one other visitor was very infrequently observed on the flowers, a syrphid of the genus *Melangyna*. *H.longirostris* is a small fly (about 7 mm long) and has a characteristically bubble-shaped abdomen with a small head. It has the peculiar habit of only visiting flowers of *M.goyenii* during the period between about 4.30 pm and 6.00 pm. The entire tube is entered and the whole body of the fly contacts the anthers as it pushes to the bottom of the tube to get to the nectar. The stigma is contacted first as the insect lands on the flower. Frequent flights between plants are made although usually several flowers are visited on one plant before moving on.

M.albosericea

This species is known only from one site on the Dunstan Range in Central Otago (grid ref NZMS1 S133 140 683). It occurs as cushions on very exposed fellfields (Johnson and Robertson 1986).

The plants form quite large cushions up to 400 mm across. Its habit is not unlike *M.colensoi*. However it differs in having very densely-hairy silvery leaves and by having erect but fairly short inflorescences. The funnel-shaped bright yellow flowers (Figure 12) are borne on these stems in January but the length of the flowering period is not known. The flowers are similar to those of *M.goyenii* in shape but are slightly smaller. They have partly fused corolla lobes like those of *M.goyenii* and is otherwise similar in morphology. The stigma is well exserted suggesting that a pollinator is required to achieve pollination.

A trip to the site in 1987 at the end of the flowering period indicated that seed set was very low. It was suspected that the pollinator may have become extinct in recent years. A further trip was made in 1988 during peak flowering. A variety of unidentified small beetles and weevils were found in the flowers. These beetles may have been pollinating the flowers, but many of the beetles made very few movements, however, and they may have been using the flower for shelter. One native solitary bee was caught on the flowers however which was not identified. The bee if it is all frequent, may be more important than the beetles, as it may cause more effective pollination. This effectiveness of either group has not been examined and it is not known whether other insects visit the flowers. It is possible, however, that the low seed set observed in 1987 may be caused by a lack of effective pollinators.

A DISCUSSION OF THE TRENDS OBSERVED IN THE FLORAL BIOLOGY OF *MYOSOTIS* SPECIES.

Interest in the flowers and pollinators of New Zealand plants has a long history. Wallace (1876, 1880) wrote at some length on the nature of the flowers and pollinating fauna. He suggested that New Zealand was characterised by a paucity of insects and that as a result, the flowers have evolved to be small and inconspicuous. Since then, various reviews have been made of the general features of New Zealand flowers and their pollinators (Thomson,GM 1881, 1927, Heine 1937, Primack 1978, 1983, Godley 1979, Lloyd 1985). The general outcome of these reviews and surveys has been that, as Wallace pointed out, the New Zealand flora is characterised by flowers that are simple in structure, non-showy, small and often white-flowered and with a strong tendency for separate sexes. Moreover, these features of the flora can, at least in part, be explained with reference to the pollinating fauna as originally suggested by Wallace. However, rather than a paucity of flower visitors as such, the studies of the work of Thomson, Heine and Primack especially have emphasised that the paucity of the New Zealand fauna is in the number of *specialised* pollinators. In particular, several important groups of specialised pollinators such as hawkmoths, birds, butterflies and long-tongued bees are rare or absent. Other groups of pollinators such as diptera, moths and beetles, however are common. The importance of the diptera in particular has been emphasised and it has been suggested that flies have evolved in New Zealand to fill niches usually occupied by bees (Thomson 1881; Heine 1937). It is generally thought that pollination by diptera and coleoptera requires a generalised simple flower morphology (Heine 1937, Primack 1978). In addition, Primack (1978) has suggested that the variability in flower visitors to a single species as conditions change will select against any specialisation that precludes one or more groups of these visitors. All of the general surveys of plant-pollinator interactions have revealed very few plant species that seem to rely on only one group of visitors.

The discovery of relatively specialised relationships of various species of *Myosotis* demonstrated here are therefore of special interest in a New Zealand context. In the five species studied in detail (leaving aside the regularly selfed species (see Chapter 2 for details), four appear to be visited primarily by a single species of visitor. This specialisation involves in each case, a

different order or family of insect. Two species have acquired dipterans as the main pollinator, one a noctuid moth, another an ant and yet another utilises beetles. In one sense this mirrors the variability of visitors suggested by Primack, but involves variation between species rather than among a single population. Speciation, it appears, has proceeded in different directions utilising different lines of pollination. The northern hemisphere characterisation of a genus as being always lepidopteran-pollinated or bee-pollinated, for instance, clearly does not apply in this country even where specialised relationships may have evolved.

It is possible that the presence of other specialised plant-pollinator relationships may exist in other New Zealand plants. The approach of most workers investigating the pollination of New Zealand plants has been one of a survey conducted in one area of as many species of plants as were available. Each species is by necessity watched for a relatively short time. Such an approach is bound to give results for plants that are regularly visited by a wide array of visitors. A casual observation of two of the species listed here (*M.goyenii*, and *M.macrantha*) would have, in all likelihood, resulted in the failure to observe any visitors to the flowers. The specialised visitors discovered here by persistent observation would have almost certainly been missed. Nevertheless, despite this bias, the general finding of plants with simple flowers visited by a wide and variable range of visitors is probably characteristic of most of the dominant New Zealand plants. Many groups of plants, however, particularly those of forest environments, have never been examined for details of their pollination biology. Studies of floral biology of New Zealand plants have almost invariably concentrated on aspects of the breeding system (Lloyd 1985). More work on the pollination aspect of New Zealand plants is clearly needed. Moreover, some attempt should be made to determine the effectiveness of each of the visitors noted on the flowers of different species. Very few detailed studies of this type have been conducted on New Zealand plants (but for example, see Primack 1979, Primack and Lloyd 1980, Bocher and Philipp 1985).

The demonstration of the involvement of ants in the pollination of one species is of interest. Ant pollination in general is rare (Faegri and van der Pijl 1971). However it may be of

interest to note that Primack (1983) also found ants visiting two species of riverbed plants - *Muelenbeckia axillaris* and *Pimelea sericeo-villosa*. The prostrate nature of many riverbed plants may be particularly suitable to visitation by ants and these insects may therefore be important in this habitat.

The occurrence of moth-pollination in *M.macrantha* is also surprising. The purple-brown-yellow flowers are not the usual colour of moth-pollinated plants, which in general are white (Faegri and van der Pijl 1971). Considering the paucity of coloured flowers in the New Zealand flora (Godley 1979) it is perhaps rather ironic that the situation where white flowers were expected (night pollination), coloured flowers are found! The discovery of moth-pollination in the purple-flowered *Pittosporum* (Godley 1979) is perhaps more than a coincidence. In addition, the close relationship of *P.huttoni* with *M.colensoi* indicates that specialisation does occur in white-flowered plants (cf Wardle 1978). More records of the colour of flowers of other plants and the type of visitor would be of great interest.

In summary, *Myosotis* appears to have diverged widely in flower syndromes within New Zealand. Specialisation along several different lines of pollinator and flower syndromes is evident. Some species have tended towards autogamy (Chapter 2) while others have developed a very close relationship with a single species of insect visitor. The diversity may have its roots in the long history of the group in New Zealand and the diversification to fill new niches and co-evolve with specialised pollinators in the post-mountain building era.

CHAPTER TWO

HERKOGAMY, DICHOGAMY AND SELF-POLLINATION IN SIX SPECIES OF *MYOSOTIS*

Herkogamy and dichogamy, respectively the spatial and temporal separation of anthers and stigmas, are two features of floral morphology that influence the parentage of seeds in cosexual plants. The magnitude of these parameters, alone or in combination, determines the frequency of self-pollination, and in self-compatible plants, the frequency of self-fertilization. Cosexual plants that avoid self-pollination usually display one or both of the features. On the other hand, incomplete separation of the pollen and stigmas allows self-pollination to occur. Herkogamy and dichogamy have traditionally been seen entirely as mechanisms to promote this avoidance of self-pollination. However a different, complementary function has recently been emphasised (Lloyd and Webb 1986, Webb and Lloyd 1986). These authors suggested that the avoidance of interference between pollen presentation and deposition and the efficient functioning of pollination may also be an important selective force.

Webb and Lloyd (1986) also described the types of herkogamy found in flowering plants. They distinguished ordered and unordered herkogamy, recognizing the latter by the fewer and more ordered contacts between pollinators and stigmas. Within ordered herkogamy, they further recognized approach herkogamy where the stigma is usually contacted before the anthers, thus rendering self-pollination from the same flower less likely. This type of flower is very common, especially amongst the more advanced angiosperms, and often requires a more specialised pollinator to operate it.

Among aspects of dichogamy that Lloyd and Webb (1986) recognized as important to the breeding system of a plant are the degree of synchrony within the plant and the degree to which the dichogamy is complete within a flower. Protogyny (the presentation of the stigma

before the pollen) is less common than protandry (the presentation of the stigma after the pollen) in animal-pollinated plants (Thomson,GM 1881, Lloyd and Webb 1986). However, they argued that when the avoidance of self-pollination is the selective force for dichogamy, incomplete protogyny rather than incomplete protandry is more likely to evolve as it provides better protection from self-fertilisation.

Meanwhile, a burgeoning body of theory on the sex allocations of cosexual species has shown that investment in the male function depends on the shape and height of the male fitness curve (Charnov 1982, Ross and Gregorius 1983, Lloyd 1984). A fitness curve describes the contribution that a structure makes to the fitness of the individual producing it as expenditure on the structure increases. In a regularly self-fertilising species, the optimal investment in male structures is small, as most of the plant's fitness is derived from seeds (Charlesworth and Charlesworth 1981, Lloyd 1987). The theoretical investment in structures primarily associated with pollination also decreases (Lloyd 1987). Lloyd also pointed out that the mode of self-pollination is important. In the extreme case, when all the progeny result from autonomous pollination without any chance of cross-pollination, allocation to the male function and pollination should be the minimum possible. Where self-pollination occurs after some delay, or when only a fraction of the ovules are immediately self-pollinated, an increased expenditure on these structures is predicted. Finally, where self-pollination can only occur by geitonogamy (ie pollen comes from another flower on the same plant), allocations to the male function should be identical to those of a plant that is obligately outcrossing.

This chapter describes the patterns of herkogamy and dichogamy in six related species of *Myosotis*, together with features of their breeding systems. It also examines the relationships between the dependence on self-fertilisation and investment in attraction and pollen. Where possible, differences among individuals of the same population will be examined to indicate whether there is adequate variation in the population to allow selection for the characters that control their breeding system.

DESCRIPTION OF THE GENERAL CONDITION

As discussed in the last chapter, it is possible to recognize several basic floral forms in the species of *Myosotis*, one of which is the tube-flower. In addition to the general features of the narrow tube and the wide rim, these flowers have other features in common that influence their floral biology, particularly the relative chances of self- and cross-pollination. The five anthers are epipetalous and dehisce longitudinally along the inner surface. The single stigma is held aloft by the style that extends up the middle of the tube. As the flower ages the tube extends, carrying the anthers with it. At the same time, the style also elongates, but to less extent. Depending on the initial starting positions of the stigma and anthers, there are three possible sequences. (1) If the stigma is exserted sufficiently far beyond the anthers, it may never be overtaken by the anthers and thus the flower is unable to autogamously self-pollinate. (2) The stigma may initially lie beyond the anthers but is later overtaken, allowing the possibility of later self-pollination. (3) The anthers may already be adjacent to the stigma when the flower opens and so the flower is able to self as soon as pollen is dehisced. As shown below, all three situations exist in species of *Myosotis*. The variation in anthers and stigma positions allows flexibility in the mode of pollination in this group.

METHODS

Taxa and field sites

Six species of *Myosotis* were selected for intensive investigation of herkogamy and dichogamy in an attempt to describe the range of herkogamy assessed to be present within the tube-flower group (see Chapter 1). One population of each species was studied in the field. The taxa chosen and the location of the populations are as follows:-

M.colensoi (Kirk) Macbride
Location: Castle Hill Basin, Broken River, Canterbury, N.Z. Grid reference: NZMS1 S66 195943
Collection date: 18.11.86
Voucher: 86005

M.uniflora Hook.f.

Location: Tasman River, near the terminal of the Tasman Glacier, Canterbury, N.Z. Grid reference: NZMS 1 S79 809 329
Collection date: 8.11.88
Voucher: 86015

M."lyttletonensis"

(Described as a variety of M. australis R.Br. by Laing and Wall, 1924 from Mt Pleasant, Lyttleton. I consider that it is certainly a distinct species, though it has yet to be given a new combination at this level).
Location: Purau Bay, Banks Peninsula, Canterbury, N.Z.
Grid reference: NZMS 260 N36 917 292
Collection date: 18.10.86
Voucher: 85027

M.forsteri Lehm.

Location (for all except seed set in the glasshouse): Poolburn Reservoir, Central Otago, N.Z. Grid Reference: NZMS 1 S144 505 403
Collection date: 19.1.86
Voucher: 96013

Location (for seed set in the glasshouse): Greenland Reservoir, Central Otago, N.Z. Grid reference: NZMS 1 S144 317 221
Collection date: 10.4.86
Voucher: 86059

M.spathulata Forst.f.

Near Sign of the Packhorse, Banks Peninsula, Canterbury, N.Z. Grid reference: NZMS 260 N37 850 234
Collection date: 19.9.88
Voucher: 88001

M."minutiflora"

(Previously described as a variety of M.pygmaea Col. by Simpson et Thomson, I consider that this taxon is worthy of specific status, but no combination has yet been made).
Location: Lake Lyndon, Porter's Pass, Canterbury, N.Z.
Grid Reference: NZMS 1 S74 186 865
Collection date: 15.3.89
Voucher: 85015

Spatial arrangement of parts

During the flowering period of each species, flowers were randomly collected from a variable number of plants from the field. An exception to this was M."minutiflora", for which

seedlings were collected in the field and the plants grown on to flowering in a glasshouse. The number of plants sampled varied according to the number available in the populations. Flowers were sampled from each of the sexual stages recognized (female, male and post-presentation) and fixed in FAA. They were later examined under a stereoscopic microscope and the lengths of the various parts were measured. A herkogamy index, the difference between the length of the style and the position of the center of the anthers, was calculated. This difference is expressed as a proportion of the style length, and is either negative, if the style is shorter than the height of the anther centres, or positive if it is longer.

A two-way ANOVA was performed with relative herkogamy as the dependent variable and the sexual stage and species as independent variables. This is a model I anova and therefore the treatment MS and interaction MS were divided by the error MS to obtain the appropriate F-ratio. Computations were done with the GLM procedure of SAS, using type III sums of squares (SAS Institute 1985). This package was used to perform all the ANOVAS and ANCOVAS mentioned throughout this chapter. To compare the sizes of the unit of attraction, a one-way ANOVA was performed on the log10-transformed size of the petal lobe for each species. Tukey's Studentised Range test was used to compare means as unequal sample sizes were used (Sokal and Rohlf 1981).

Seed production

The number of seeds produced per fruit was counted for a number of naturally occurring plants which were untreated and therefore open-pollinated. There are four ovules per flower, so maximum seed set occurs when each fruit produces four single-seeded nutlets. As the nutlets are easily observed in the mature calyx, it was not necessary to remove fruit to estimate seed set.

Seed production was also measured after pollinators were prevented from visiting the flowers, either by growing the plants in an insect-proof glasshouse or by covering them with netting in the field. The seed produced in these circumstances is the result of autogamous selfing. I was unable to obtain data for *M.uniflora* because this species does not survive under

cultivation and it is impossible to exclude the main visitors, ants, from visiting with netting in natural populations. The population of *M.forsteri* cultivated in the glasshouse was not the same as the one for which seed set was measured in the field. The two locations are close however (see study sites above). In addition the only measure of seed set in M."minutiflora" was collected from a single glasshouse-grown plant.

A two-way analysis of variance was computed to compare seed set in the field and glasshouses and to compare species. The interaction between species and "site" are significant (see results below), so no interpretation of the single factor results was attempted (Sokal and Rohlf 1981). Instead, two one-way Anovas were performed to compare species separately at each "site". For each Anova, Tukey's studentised range tests were performed to compare seed set between species. In addition, t-tests were carried out to compare seed set between sites within each species. Not enough data was available for *M.uniflora* and M."minutiflora" to be included in these analyses.

Duration of Sexual Stages

In order to estimate the time a flower spends in each of the sexual stages, "static" samples were made. For these, the number of flowers in each of the recognized stages was assessed for a number of plants observed during the at one time. The mean proportions of flowers at each phase were determined and expressed as percentages of the total. This method would be subject to error if flowers opened all at the same time and went through their life-cycle synchronously. However, in *M.colensoi* and M."lyttletonensis" flowers were found to open at all times of the day (unpublished data), and in the other species flowers at all stages occur on the plant at the same time. Hence there is no evidence of synchronous opening. This method gives information on the relative times in each phase rather than the actual times. A one-way ANOVA on the duration of the female phase was carried out with the species as the independent variable, and the species means were compared with the Tukey Studentised Range Test.

Pollen:Ovule Ratios

Pollen:ovule ratios were estimated by two methods.(1) Where there was less than 1000 pollen grains per anther, a whole anther (M."minutiflora", M."lyttletonensis", *M.forsteri*) or the whole flower (*M.spathulata*) was softened with 5% NaOH for several minutes and then squashed under a coverslip on a slide. The number of pollen grains on the whole slide was then counted. This total was multiplied by five (the number of anthers), except where the whole flower had been counted, and divided by four (the number of ovules) to give the pollen:ovule ratio. (2) Where there was more than 1000 grains per anther, the whole flower (M.uniflora) or a single anther (*M.colensoi*) was softened in 5% NaOH, squashed and rinsed through a sieve with distilled water. The wash was centrifuged at 3000 RPM for 5 minutes and decanted. The grains were re-suspended in 0.1 ml of a mixture of 3:1 Lactic acid and Glycerol (Lloyd 1965) and transferred to a haemocytometer. The haemocytometer is marked in two areas into 9 squares of 0.0001 ml. For each suspension, 18 counts each of 0.0001 ml were made, and the mean of these was multiplied by 1000 to estimate the total number of grains in the suspension. This was replicated with flowers from different plants a variable number of times. The pollen-ovule ratio was calculated as for method 1 above.

A one-way anova was performed on the log10-transformed pollen:ovule ratios to compare species. Again Tukey's Studentised Range Test was used to compare means.

Within-Species Variation in Herkogamy

Although this study was primarily designed to measure differences in herkogamy and associated characteristics among species, for three of the species (*M.uniflora*, M."lyttletonensis" and M."minutiflora"), the data was collected in such a way that differences between plants of the same population could also be assessed. For these species, one flower (two for *M.uniflora*) was collected for each stage from each plant. Because herkogamy changes with the stage of the development of a flower, the measure for each flower needs to be adjusted for this time difference in order to be used as a replicate for that plant.

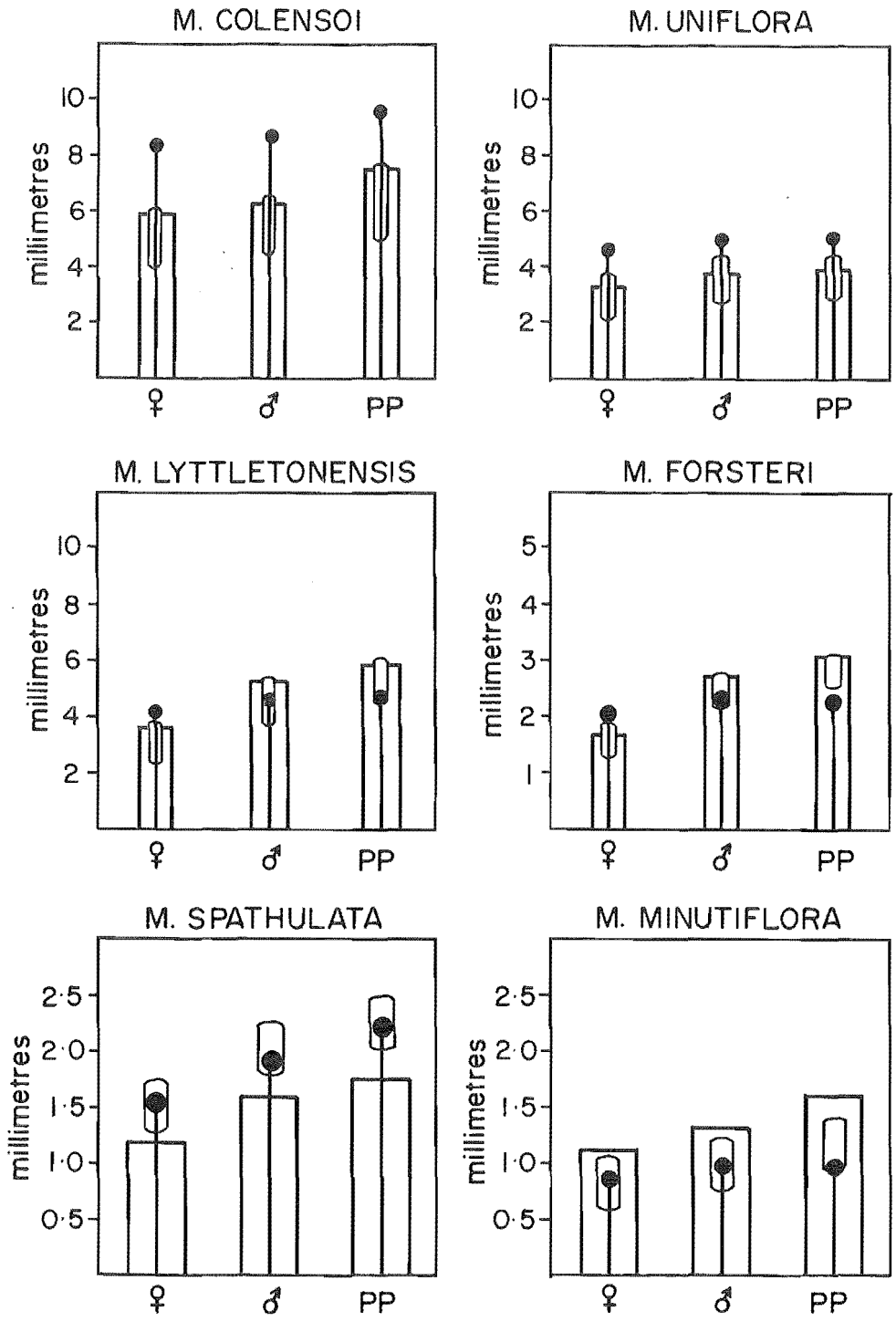


Figure 1. The development of floral parts over time in the six species studied. The positions of the stigma, anthers and the length of the floral tube are indicated by solid circles, narrow rounded blocks and broad oblongs respectively. Fem = female phase; PP = post-presentation phase.

An analysis of covariance was used to test whether plants differed in the degree of the herkogamy across the life-time of the flower. The age of the flower (estimated by measuring the proportion of the maximum total flower length reached on that plant) was therefore used as a covariate to adjust the estimate for herkogamy. If a plant has consistently less herkogamy than another its estimated adjusted herkogamy will be lower. In order to examine this variation further, the correlation between style length (similarly adjusted for flower age) and herkogamy was tested.

RESULTS

Spatial arrangement of parts and herkogamy

Figure 1 shows the size and relationships of parts in each of the six species chosen to illustrate the range of conditions in the tube-flower group. It is clear that the species display wide differences in herkogamy, particularly in whether the anthers reach the stigma and the time at which they do so. In *M.colensoi* and *M.uniflora*, the stigma always protrudes further than the anthers, ie they show "approach herkogamy", throughout the life of the flower. It therefore appears that a pollen vector is required to transport pollen to the stigma. These species are referred to as "outcrossers" though of course the mechanism does not prevent geitonogamous self-pollination. It merely requires that a pollen vector to move pollen to the stigma, ensuring that there is at least a chance of outcrossing. In M."lyttletonensis" and *M.forsteri* the stigma is initially exserted beyond the anthers but is overtaken during the male phase. Once this has occurred, pollen can be freely deposited on the stigma in the narrow tube, and thus self-pollination can occur. There is, however, a period where pollination from another flower can occur before self-pollination becomes inevitable. I will therefore refer to these as "delayed selfers". In the remaining two species, *M.spathulata* and M."minutiflora", the anthers and stigmas begin in close contact and remain so throughout the life of the flower. As soon as anthers dehisce, pollen can be immediately deposited on the stigma and self-pollination will result. These species will be referred to as "regular selfers". Further justification for these descriptive terms is provided below.

Table 1 Effect of species and sexual stage (female, male and post-presentation) on the herkogamy index (the difference between the centre of the stigma and the centre of the anthers as a proportion of the style length).

	MEAN HERKOGAMY ± S.E.(n)		
	SEXUAL STAGE		
	FEMALE	MALE	POST-PRESENTATION
M.colensoi	0.387 ± 0.007 (14)	0.352 ± 0.010 (14)	0.299 ± 0.023 (14)
M.uniflora	0.241 ± 0.015 (38)	0.210 ± 0.009 (40)	0.182 ± 0.009 (38)
M."lyttletonensis"	0.211 ± 0.036 (22)	-0.003 ± 0.032 (21)	-0.093 ± 0.029 (21)
M.forsteri	0.239 ± 0.023 (11)	-0.084 ± 0.054 (11)	-0.206 ± 0.027 (11)
M.spathulata	0.023 ± 0.028 (14)	-0.051 ± 0.023 (15)	-0.027 ± 0.020 (14)
M."minutiflora"	0.014 ± 0.036 (13)	-0.088 ± 0.057 (13)	-0.301 ± 0.040 (11)

ANOVA

Source of variation	df	S.S.	F	P
Model	17	9.01	40.60	0.0001
Species	5	6.22	95.29	0.0001
Sexual Stage	2	1.90	72.80	0.0001
Stage * Species	10	1.16	8.87	0.0001
Error	261	3.41		

If the herkogamy evident from Figure 1 is expressed as a herkogamy index (the relative difference between the position of the center of the anthers and stigma), a statistical comparison between the species can be made. The herkogamy index is shown in Figure 2 for each of the sexual stages of the six species.

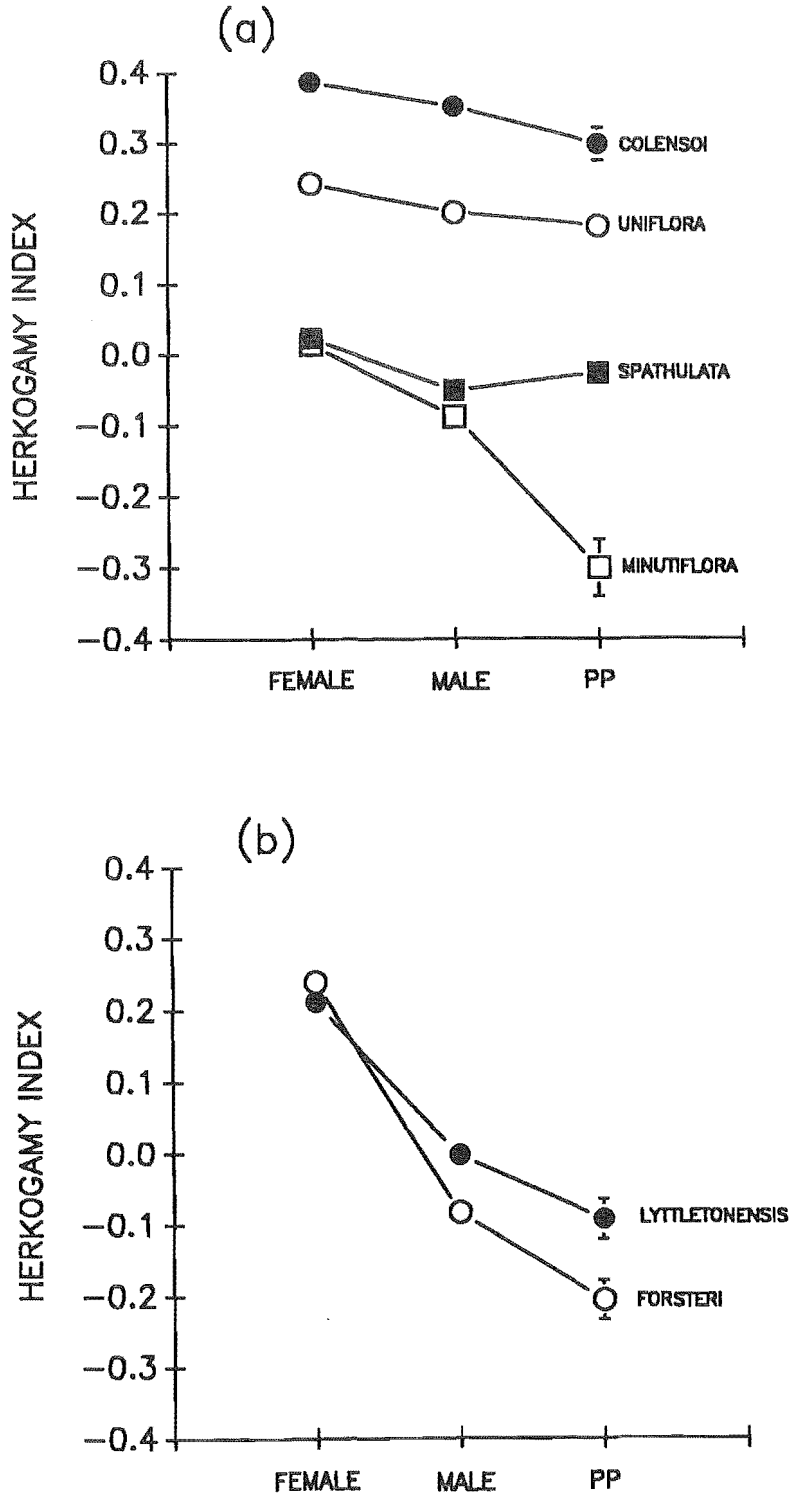


Figure 2. The herkogamy index (the height of the stigma minus that of the centre of the anthers) in three sexual stages. Standard errors are shown for the post-presentation stage only. PP = post-presentation.

a *M.colensoi, M.uniflora*, *M.spathulata* and M."minutiflora".

b M."lyttletonensis" and *M.forsteri*.

Table 1 presents the results of the two-way ANOVA and confirms the differences between the species and the importance of the stage of the flower. Both factors are highly significant. The interaction between these effects is also significant and reveals that the magnitude of the effect of sexual stage differs between the species. In particular, the two species in which the stigma is overtopped during the male phase change more over time than either the species in which the stigmas are never overtopped and the species in which the stigma are always overtopped. Because the interaction is significant, no comparisons of means were carried out.

Seed set in enclosed and open-pollinated plants

Table 2 and Figure 3 present the results of the seed set experiment. There are three main results. Firstly, seed set in enclosed plants is significantly different from open-pollinated plants in two of the four cases where sufficient data is available. *M.colensoi* sets no seed when enclosed. *M.forsteri* also sets slightly but significantly less seed in the glasshouse. Some caution is required when interpreting this result however, as different populations were measured. Moreover, the enclosed plants were grown in the glasshouse and not in field conditions. The other two species, M."lyttletonensis" and *M.spathulata* produced the same amount of seed per fruit in both situations. The different capacities for autogamous selfing leads to a significant interactionin the Anova between species and site. Secondly, seed set in open-pollinated plants (and enclosed plants) increases as the species becomes less herkogamous. Moreover, the coefficient of variation (Table 2) declines. *M.colensoi* and M."lyttletonensis" not only have lower average seed set than *M.forsteri* or *M.spathulata*, but are also more variable in the amount of seed that is set. The very different sample sizes between species, however, demands that some caution is used in comparing the variability of seed set between species.

Table 2. Seed set per fruit in open pollinated and enclosed plants. There are 4 ovules per flower.

SPECIES	MEAN SEED SET ± S.E. (N) ENCLOSED	OPEN	OPEN > ENC? (t-test, p < 0.05)	C.O.V. (OPEN)
M.colensoi	0.00^{a} ± 0.000 (5)	1.75^{a} ± 0.756 (31)	yes	2.41
M."lyttletonensis"	2.04^{b} ± 0.226 (2)*	2.28^{b} ± 0.640 (29)	no	1.51
M.forsteri	3.43^{bc} ± 0.170 (3)*!	3.93^{c} ± 0.071 (7)	yes	0.05
M.spathulata	3.67^{c} ± 0.660 (17)	3.64^{c} ± 0.413 (16)	no	0.45
M."minutiflora"	3.93 (1)			

* Glasshouse grown; ! From Little Valley, Otago
Small letters indicate means that are not significantly different from other means in the *same* column only.

Two-way Anova

Source of variation	df	S.S.	F	P
Model	7	112.8	43.35	0.0001
species	3	97.7	87.56	0.0001
site (open v enc)	1	4.68	1.62	0.2931
species * site	1	8.69	7.79	0.0001
Error	103	38.29		

M."minutiflora" excluded from the analysis

one-way Anovas

Species (Open Plants)	3	58.32	50.24	0.0001
Error	81	31.34		
Species (Enclosed Plants)	3	52.96	55.84	0.0001
Error	22	6.955		

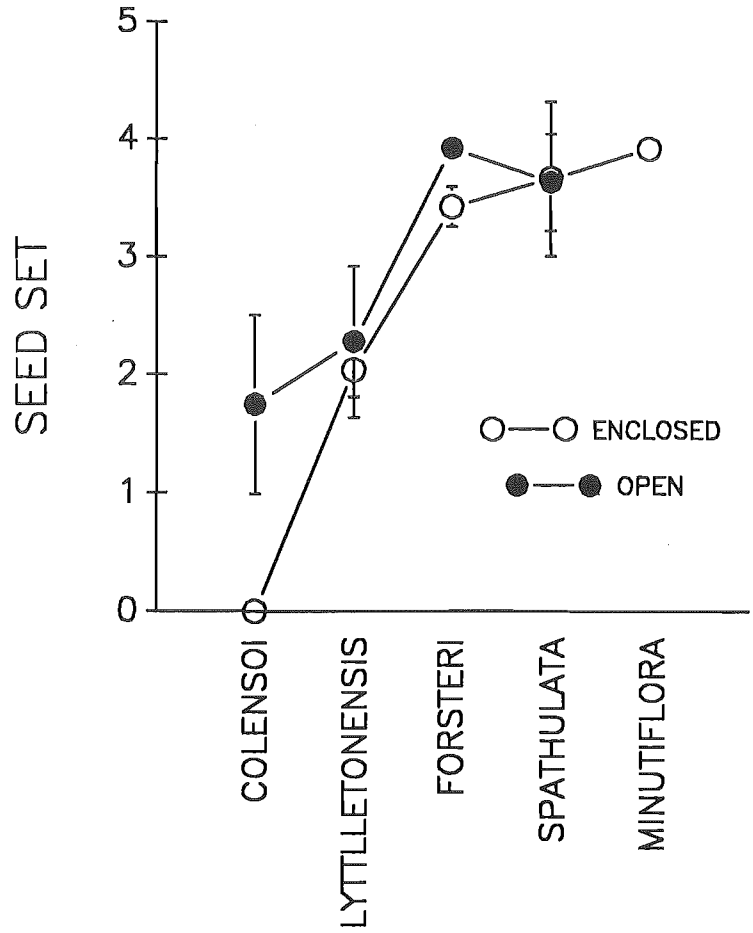


Figure 3. Seed set in open-pollinated and enclosed plants. Data for M.uniflora was not available.

Relative duration of the female phase

All the species observed (and others not shown here) have flowers that last between 2 and 5 days. The exact flower duration was not determined as it depends on the climatic conditions over the time of observation. However, by making use of the relative numbers of flowers in each of the sexual stages, one can estimate the relative time a flower spends in each phase (Figure 4). Both outcrossers have a short, strictly female phase (Table 3) which is sufficient in *M.colensoi* to allow most stigmas to be pollinated. (see Chapter 3). The delayed selfers, however, have a longer phase. Consequently, self-pollination in the male phase is relatively delayed. On the other hand, the regular selfers have a very short female phase or no period at all when only the stigmas are presented. In these species self-pollination occurs almost immediately and the chance of outcrossing occurring is almost zero.

Table 3. Female phase duration (expressed as a percentage of the total) based on "static" samples.

Duration of Female Phase	- mean % ± s.e. (n)
M.colensoi	12.1[a] ± 1.41 (17)
M.uniflora	11.2[a] ± 1.12 (38)
M."lyttletonensis"	17.7[a] ± 1.71 (17)
M.forsteri	33.3[b] ± 7.60 (19)
M.spathulata	3.1[c] ± 1.50 (61)
M."minutiflora"	0.0

Small letters indicate means that are not significantly different using Tukey's Studentised Range test.

ANOVA

Source of variation	df	S.S.	F	P
Species	4	17528.9	27.83	0.0001
Error	147	23143.4		

M."minutiflora" was not included in the analysis.

An analysis of variance of the duration of the female phase shows that the species differ significantly in the relative durations of this phase (Table 3). Comparing the means by the Tukey test shows that the two selfers, have significantly shorter female phases than the others, and that the delayed selfers have longer female phase than the two outcrossers. The difference between M."lyttletonensis" and the outcrossers is not significant, however.

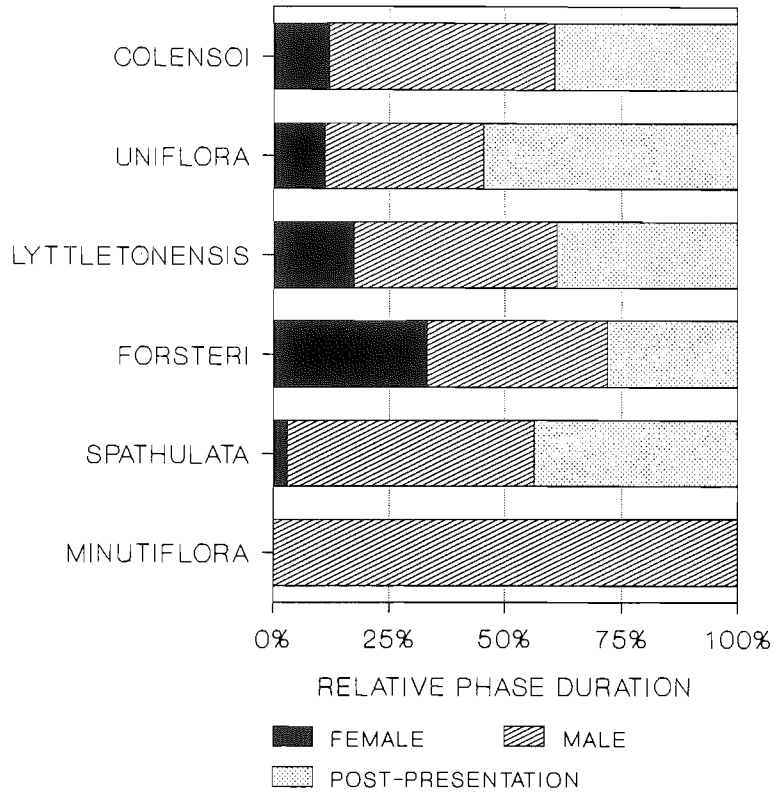


Figure 4. Proportion of flower life spent in the various sexual stages (calculated from "static" samples).

Pollen:Ovule Ratios and Flower Size

Figure 5 shows the mean pollen:ovule ratios and petal lobe size for each of the species. The species are arranged in order of decreasing herkogamy and increasing ease of self-pollination. It is clear that the species that rely on pollen vectors to achieve pollination have much higher pollen:ovule ratios. This is confirmed by an ANOVA of the ratios (Table 4). The outcrossers have much higher ratios than the delayed selfers, which are in turn significantly higher than the regular selfers.

The differences in expenditure on pollen is paralleled by similar differences in expenditure on attraction. The species that are dependent on vectors and those that delay selfing, have in general larger flowers (Figure 5b, Table 4).

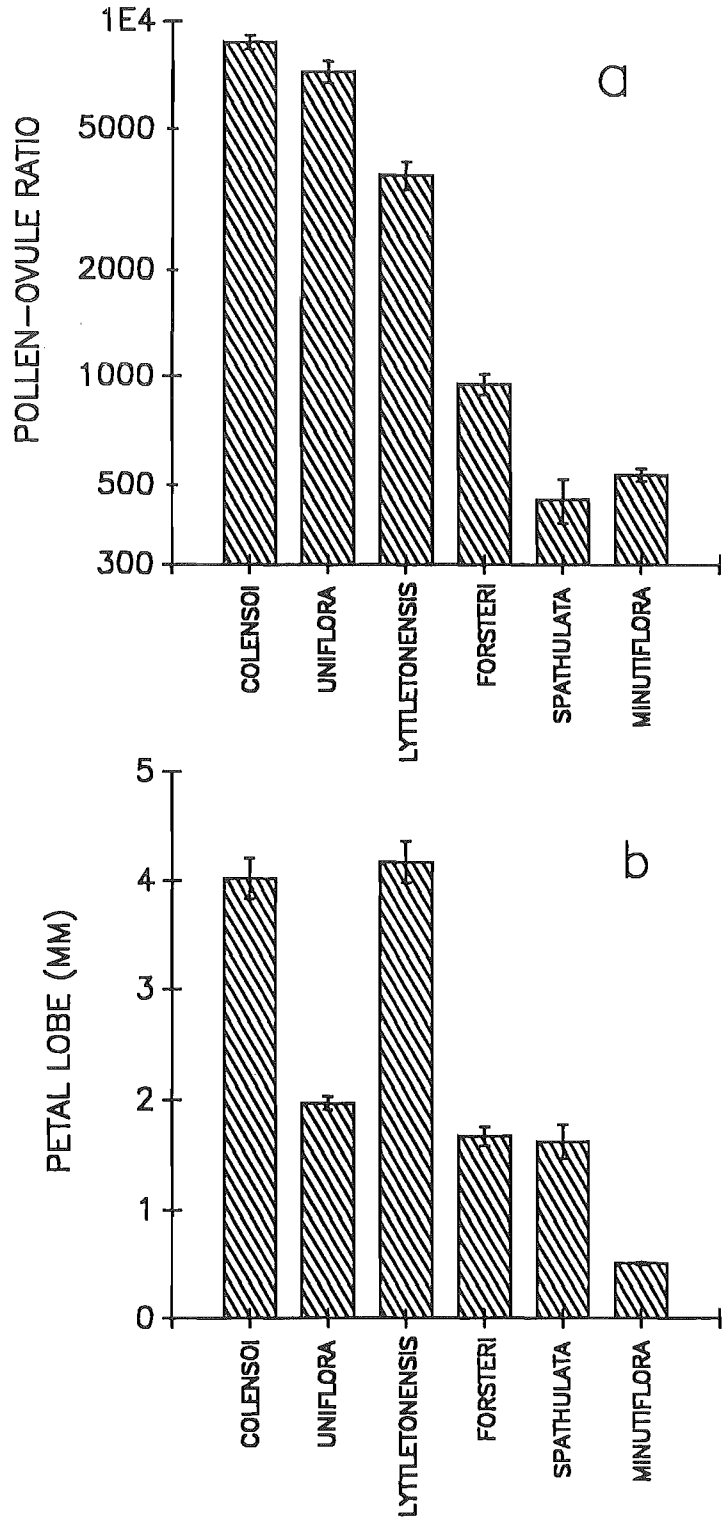


Figure 5. Pollen:ovule ratios and flower size.
a Pollen:ovule ratios (means ~ standard error) plotted on a logarithmic scale.
b Length of the petal lobe (means ~ standard error).

Table 4. Pollen:ovule ratios and petal lobe size (at the post-presentation phase).

	P:O RATIOS MEAN ± S.E. (n)	PETAL LOBE (mm) MEAN ± S.E. (n)
M.colensoi	8768^{a} ± 398.5 (10)	4.03^{a} ± 0.187 (14)
M.uniflora	7237^{a} ± 504.4 (26)	1.97^{b} ± 0.058 (20)
M."lyttletonensis"	3711^{b} ± 330.5 (24)	4.17^{a} ± 0.190 (21)
M.forsteri	$947{\cdot}7^{c}$ ± 62.7 (10)	1.67^{b} ± 0.084 (11)
M.spathulata	454.1^{d} ± 63.6 (7)	1.62^{b} ± 0.036 (14)
M."minutiflora"	533.6^{d} ± 20.5 (5)	0.51^{c} ± 0.012 (12)

Small letters indicate means that are not significantly different using Tukey's Studentised Range test.

ANOVA

Source of variation	df	S.S.	F	P
$\log_{10}$ Pollen-Ovule Ratios				
Species	5	16.04	171.50	0.0001
Error	76	1.42		
$\log_{10}$ Petal Lobe Size				
Species	5	7.74	420.44	0.0001
Error	86	0.0037		

While it seems that expenditure decreases rapidly in pollen allocation and somewhat less rapidly in flower size as the ease of self-pollination increases, there is little difference in the size of the seeds of these species (pers obs). It is apparent therefore, that the ratio of expenditure on pollination costs (pollen and attractive structures) and seed allocation declines as the species become more frequently self-pollinating.

Within-species Variation in Herkogamy

There is considerable variation among related taxa in the degree of herkogamy. We now enquire whether variation in herkogamy also exists within a population. There is some data to test this for three of the species studied - *M.uniflora*, M."lyttletonensis", and M."minutiflora". Table 5 presents the results of the analysis of covariance, where it is possible to partition the variation due to the developmental stage of the flower from that due to plant effects. This allows between-plant variation to be considered, although the number of replicates per individual plant is relatively small (3-6). Nevertheless, for two of the species, *M.uniflora* and M."lyttletonensis", there are significant differences between plants in the degree of herkogamy they exhibit. This variation can be displayed as a frequency distribution of the mean adjusted herkogamy in each plant (Figure 6). The relative amount of herkogamy displayed depends on the species, as we have seen previously. Nevertheless there is a considerable amount of variation between individuals especially in M."lyttletonensis" (Table 5). This variation is important as it reflects variation in the time at which anthers and stigmas will come in contact and hence when self-pollination will occur. For this species we can see from Figure 6c that this variation in herkogamy is positively correlated with style length. It therefore seems that the difference between plants is associated with a difference in style length.

M.uniflora also shows variation in herkogamy although it is more consistent between individuals than in either of the other species. The relationship with style length is positive but not significant. M."minutiflora" shows no consistent variation between individuals. However, with the limited number of samples taken per plant there may be plant differences that remain undetected.

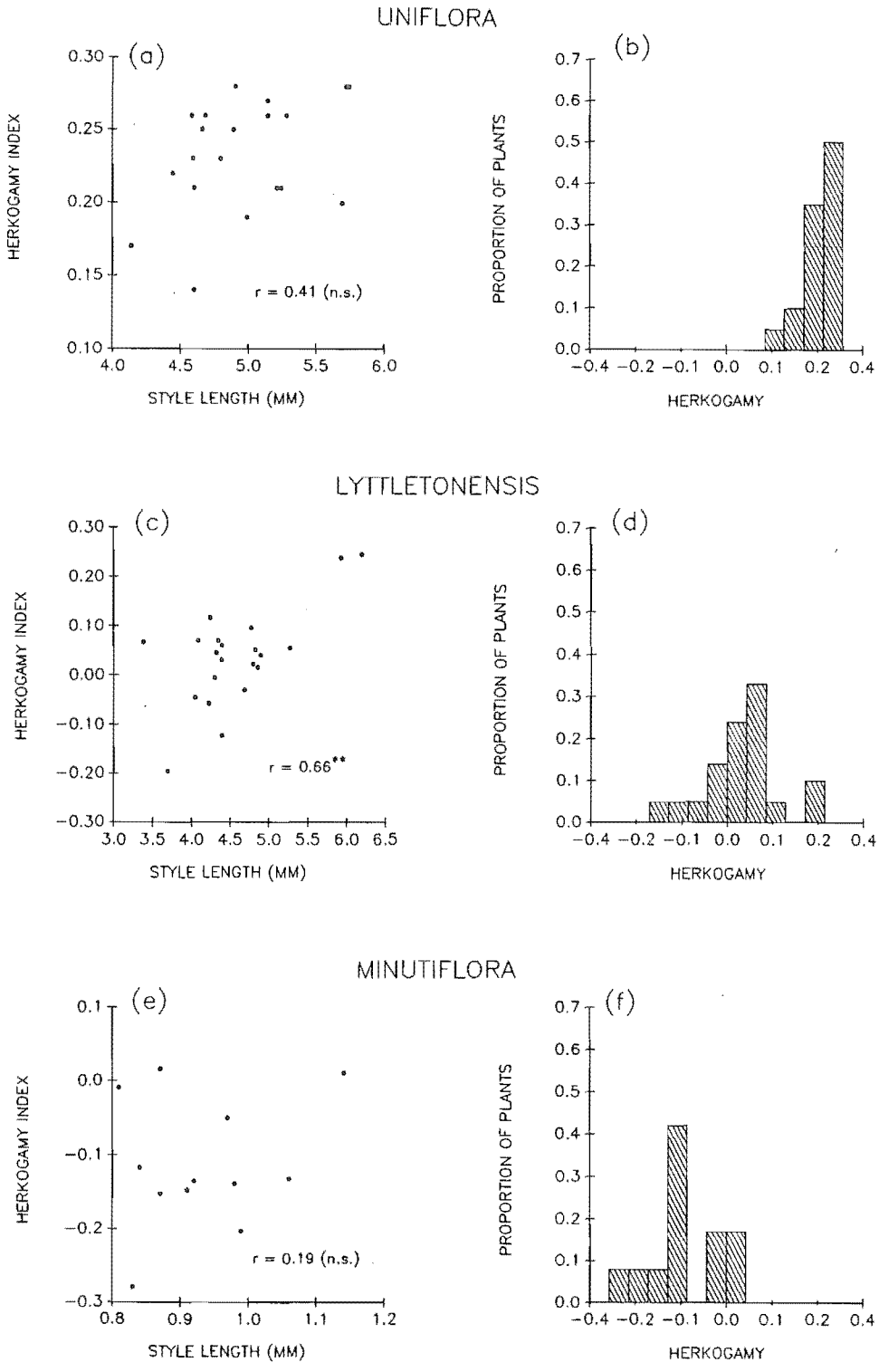


Figure 6. Intraspecific variation in herkogamy.

a,c,e Relationship between the herkogamy index (mean adjusted for flower age (see text)) and style length.

b,d,f Relative frequency of herkogamy.

DISCUSSION

By utilising an unusual mechanism of combined herkogamy and dichogamy, the six species examined in this chapter are able to achieve widely varying levels of dependence on autonomous and vector-requiring pollination mechanisms. It has been possible to group the species into three broad strategies. (1) Outcrossers - *M.colensoi* and M.*uniflora* (2) Delayed selfers - *M.forsteri* and M."lyttletonensis" and (3) Regular selfers - *M.spathulata* and M."minutiflora".

The first group require a pollinator in order to produce any seed and the pollen must come from a different flower. However, this strategy cannot prevent geitonogamy, as the flowers within plants are asynchronous in their flowering. At any one time there are usually flowers in all sexual stages, so a pollinator visiting several flowers of that plant in succession can transfer pollen onto receptive stigmas of the same plant. The second and third strategies both result in autogamous self-pollination if the flowers are not visited, but in the delayed selfers there is an initial opportunity for a pollinator to visit before autonomous self-pollination is possible.

The degree of stigma exsertion has often been assumed or shown to affect the amount of autogamous selfing within races of one species (Lloyd 1965, Moore,DM and Lewis 1965, Rick et al. 1977 but see (Schoen 1982) or between related species (Vasek 1958, 1964, 1965, Rollins 1963, Garnock-Jones 1976, Spira 1980). The shift from outcrossing to delayed selfing and finally to regular selfing is able to be achieved by the relatively simple modification of a change in style length. Style length has been shown to vary consistently between individuals within a population in two of the species (*M.uniflora* and M."lyttletonensis") and is presumably under genetic control.

Table 5. Analysis of Covariance of herkogamy with the age of the flower (estimated by the proportion of final size) as co-variate.

Source of variation	df	S.S	F	P
M.uniflora				
Model	20	0.5564	7.67	0.0001
Plant	19	0.2250	3.20	0.0001
Age (cov)	1	0.3357	92.49	0.0001
Error		134	0.486	
M."lyttletonensis"				
Model	21	2.032	18.05	0.0001
Plant	20	0.600	5.60	0.0001
Age (cov)	1	1.078	201.05	0.0001
Error		41	0.220	
M."minutiflora"				
Model	12	1.123	5.82	0.0002
Plant	11	0.2456	1.39	0.2458
Age (cov)	1	0.6824	42.46	0.0001
Error		22	0.3536	

The Ancovas explain 53.3%, 90.2% and 76.1% of the variation in herkogamy for *M.uniflora*, M."lyttletonensis" and M."minutiflora" respectively.

Dichogamy and herkogamy are usually considered separate mechanisms to encourage cross-fertilisation, but it is clear that in *Myosotis* (and some other gamopetalous dicots), their operation is interrelated. The combination of herkogamy and dichogamy in a way that allow such flexibility in breeding systems has previously been inadequately appreciated. Examples of plants that utilise a delayed self-pollination mode by a variety of mechanisms, are given in (Kerner 1902, Knuth 1906-1909, Faegri and van der Pijl 1971) and delayed selfing may be frequent in

self-compatible plants. Müller (1883) described the delayed overtopping of the stigma by the anthers in *Myosotis versicolor* (*discolor*) and a similar arrangement is found in some species of Euphrasia (Kerner 1902, Barker 1982), some members of the Gentianaceae and Scrophulariaceae, and is very common in the order Solanaceae (Kerner 1902). It has not been previously adequately demonstrated however, that this strategy allows divergence in breeding systems among related species, and that such divergence is made possible by the relatively simple change of an alteration of style length. The system depends on the filaments being fused to the corolla and therefore is restricted to gamopetalous species. It may, however, be more common than is appreciated in such species.

Protogyny is the exception rather than the rule in animal pollinated plants. When it does occur, it is frequently associated with some sort of specialised floral mechanism such as trap blossoms that require protogyny in order to operate. In this case, it is clear that the mechanism of delayed selfing depends on the flowers being incompletely protogynous. The mechanism of delayed selfing shown in *Myosotis* may well be reasonably common and, along with other delayed selfing mechanisms, help to explain the anomalous protogyny in this and other genera.

The data on seed set in the open-pollinated plants and enclosed plants confirm the predictions made on the relative ease of selfing from the herkogamy index. No data is available for *M.uniflora* but *M.colensoi* shows the effectiveness of its strong herkogamy in preventing autogamy. Differences in the mean and variability of seed set in open-pollinated plants are also apparent. In the selfing species high seed set is assured. M."lyttletonensis" is again intermediate however, suggesting that individual plants of this species differ in their ease of self-pollination. Other similar patterns of higher reproductive assurance and lower variability in seed set is frequently found in autogamous plants (Ornduff 1969).

Although I have not directly measured the rate of selfing in these species, except for *M.colensoi* (see Chapter 1), it seems highly likely that the differences observed in herkogamy do indeed lead to differences in the naturally occurring selfing rate.

Theory predicts that as the mode and frequency of selfing changes, so too will the relative allocations to the male function and to pollination mechanisms. Such decreased allocations in selfing species have been shown several times by pollen:ovules ratio (Lloyd 1965, Cruden 1977, Lord 1980, Philbrick and Anderson 1987); and allocations to the androecium (Schoen 1984, Svensson 1988) Attractive structures (petals, corollas) are also often smaller in selfing species (Grant,V 1954, Vasek 1958, 1964, 1965, Rollins 1963, Lloyd 1965, Arroyo 1975, Garnock-Jones 1976, Rick et al. 1977, Schoen 1982). The pattern in *Myosotis* is consistent with that observed in other genera, as pollen:ovule ratios and flower size decrease as the mode of pollination moves toward autogamy. Delayed selfers are intermediate in these characters, as theory predicts (Lloyd 1987), except that M."lyttletonensis" has a corolla size that is larger than expected. This may be because the seeds are bigger in this species and that the ratio of flower:seed investment may therefore be smaller than it appears. In addition this species grows in a small remnant of native vegetation and seems to not be visited by pollinators despite its apparently attractive display. It may be that the style length variation present in the population indicates that an evolutionary change from an outcrossing morphology to delayed selfing is in progress, and that the expected decrease in flower size as the dependence on autogamy increases has yet to evolve.

The autogamous species *M.spathulata* and M."minutiflora" both invest little in pollen or flowers. In fact M."minutiflora" has extremely small flowers (approximately 1 mm in diameter). It would appear that these species derive very little fitness from cross-pollination and that this mode has largely been abandoned.

The relative dichogamy patterns show parallel trends to those of herkogamy. In the regular selfers dichogamy is absent. *M.spathulata* flowers spend only about 3% of the time in the female phase and M."minutiflora" no time at all. Apparently the chance of being pollinated by a visitor is so small that there is no advantage in delaying dehiscence. Similar breakdowns of dichogamy have been shown to occur with autogamy several times, although in most cases the

dichogamy evident in the outcrossers is protandry rather than protogyny (Moore,DM and Lewis 1965, Arroyo 1975, Schoen 1982). The delayed selfers, on the other hand, spend a much longer time in the female phase (33% in *M.forsteri* and 17% in M."lyttletonensis"). It seems that in these species there is an advantage in delaying the onset of the male phase in order to maximise the chance of pollination occurring before anther dehiscence.

While it is apparent that the lack of herkogamy and dichogamy in the regular selfers acts to ensure that autogamous selfing is successful, it is more problematical to assume that the presence of such mechanisms in the outcrossers has evolved to prevent self-fertilization. Interference between the male and female functions has been postulated as being another potential selective force for the evolution of stigma and pollen separation (Lloyd and Webb 1986, Webb and Lloyd 1986). Because both forces are likely to select for similar traits, it is difficult to separate which is the more important. However, Lloyd and Webb made certain predictions about the nature of the herkogamy and dichogamy that can be examined here. They argued that dichogamy and herkogamy are frequently associated with self-incompatabilty and that therefore the separation in time and space is not necessary to prevent self-fertilization. However M.colensoi, at least, is fully self-compatible (Chapter 1). Approach herkogamy is probably equally effective in preventing self-pollination and self-interference. They also predicted that "whenever the avoidance of self-fertilisation is the selective force responsible for dichogamy, one expects incomplete protogyny rather than incomplete protogyny to be selected" (see also Webb 1981). In Myosotis the flowers are incompletely protogynous and in the outcrossing species and delayed selfers are, at least initially herkogamous. It would appear, therefore that the presence of herkogamy and dichogamy in the outcrossing group may, in this case, have evolved to avoid self-fertilisation rather than self-interference, although as both forces act in complementary ways, the importance of self-interference as an additional selective force cannot be eliminated. In addition, if the outcrossing species have evolved from delayed selfers, as indeed is likely (see Chapter 1), it would seem probable that they inherited protogyny as this is necessary for the delayed selfing mechanism to function.

In conclusion, it seems evident that by a relatively simple change in the length of the style it has been possible for *Myosotis* to evolve a variety of breeding systems. Within this group of related species, there are examples of obligate selfers, delayed selfers allowing cross-pollination but not depending on it, through to species which a pollinator is required to achieve any seed set. Coupled with the change of style length there has been an associated change of resource allocations with a decreasing allocation to pollen and pollination mechanisms as the frequency of self-pollination increases.

CHAPTER THREE

RATES OF POLLEN DEPOSITION AND REMOVAL IN *M.COLENSOI* AND COMPARISONS OF EFFECTIVE AND NOMINAL PHASE DURATIONS

Bateman (1948) made the general observation that for oogamous plants and animals, the maternal expenditure on an offspring is usually much higher than the paternal expenditure. He postulated that maternal fitness is usually more limited by the resources available for reproduction, while paternal success is more limited by the number of offspring able to be sired. He argued that this explains how competition between males leads to sexual selection for traits that confer an advantage in maximising the number of offspring that they sire. This idea has become to be known as "Bateman's principle" and has have been of much importance in studying fitness of animals and more recently, plants (Charnov 1979, Willson 1979, Lloyd and Yates 1982, Queller 1983). Charnov (1979) argued that where the conditions of Bateman's principle holds, most gains in fitness at anthesis are made through the male function and so in hermaphrodites, selection should favour traits that improve the successful export of pollen to compatible stigmas. Despite the realized importance of the male function at anthesis, as Janzen (1983) and Wolfe and Barrett (1989) point out, few studies have addressed this side of plant reproduction . This may be because of the difficulty in measuring the rate and timing of pollen removal.

Generally, sexual selection has been invoked to explain the apparent "over-production" of hermaphrodite flowers that do not set seed (Willson and Rathcke 1974, Gilbert 1975, Willson and Price 1977, Queller 1983, Sutherland and Delph 1984, Couvet et al 1984). Some empirical data has been collected that indicates that the female function becomes saturated with relatively little effort, and that most fitness gains at anthesis are made on the paternal side (Bell 1985). Willson (1979) suggested that sexual selection may be important in explaining the evolution of other traits including sex expression, resource allocations, pollinator mechanisms and in the relative duration of staminate and pistillate phases. Lloyd and Yates (1982) argued that where

seed set is resource limited, selection will favour prolonged duration of the staminate phase and staggered presentation of pollen.

Unfortunately, the relative duration of male and female phases has been measured in ways that give ambiguous or misleading results. Phase durations are usually assessed by the physical appearance of anthers and stigmas (e.g. Garnock-Jones 1976, Schoen 1977, Schemske 1978, Willson and Schemske 1980, Bertin 1982, Lloyd and Yates 1982, Devlin and Stephenson 1984, 1985) or by the duration of stigma receptivity and pollen viability (Schoen 1977, Palmer et al 1989). However, as Lloyd and Webb (1986) point out, "what matters in nature is the time when pollen is available to be transferred from a blossom compared with the time when stigmas are capable of receiving pollen that has a prospect of fertilising ovules". Information on the period of effective presentation of surfaces is what is needed. However, this type of data is almost completely lacking in the literature.

Several workers have described mechanisms that prolong the presentation and pickup of pollen by pollinators (Lloyd and Yates 1982, Devlin and Stephenson 1984, Haynes and Mesler 1984, Lloyd 1984, Harder et al 1985, Harder and Thomson 1989). Plants that are able to spread out the time over which their pollen is removed are likely to export pollen to more receptive stigmas because more pollinators will be involved in transport (Lloyd and Yates 1982). Sib competition among pollen grains from the same parent on one pistil is thereby reduced. Moreover, Harder and Thomson (1989) show that in *Erythronium* pollen deposition efficiency is greatest when only a small amount of pollen is initially picked up, as this results in less wastage of pollen. Two strategies exist for prolonging pollen presentation. Pollen dispensing strategies involve mechanisms that allow only a portion of the pollen of a flower to be made available at one time. A variety of mechanisms that do this have been noted (Lloyd and Yates 1982, Brantjes 1983, Haynes and Mesler 1984, Devlin and Stephenson 1985). Pollen packaging strategies stagger the presentation of pollen by packaging it into small bundles (e.g. anthers) which present their pollen sequentially (Percival 1955, Harder et al 1984). Either or both mechanisms are likely to be selected whenever the conditions assumed under Bateman's principle occur i.e. adequate

pollination for 100% fertilisation and more than one visit per flower during pollen presentation (Lloyd and Yates 1982, Harder and Thomson 1989).

There has been very little study on the proximate factors that influence flower longevity and dichogamy (Primack 1985). Frequently, an incidental or anecdotal reference to the influence of weather is made (e.g. Percival 1950) but only rarely has an attempt been made to substantiate this (Wells 1988).

This chapter attempts to (1) determine the timing of pollen removal and receipt and (2) contrast these effective phase durations with the nominal phase durations (3) examine pollen packaging strategies and (4) determine the effect of weather on phenology in *Myosotis colensoi*.

METHODS

Phase preferences of pollinators

In order to assess the preference pollinators may have for flowers in either presentation phases (yellow corolla scales with stigma and/or pollen presented) or post-presentation phase (scales fading or faded, anthers usually brown) (see chapter 2), individuals of the tachinid fly *Protohystricia huttoni* (Malloch) were observed foraging on *M.colensoi*. It was not possible to further distinguish flowers in the male and female phases, as this would have required closer scrutiny of the flowers than could be managed without scaring the fly off. Flies were observed as they moved over a plant, and the number of presentation phase and post-presentation phase flowers that were visited were counted. The total numbers of flowers in each phase were also counted. The observations were made during peak flowering on 14 November 1987 and again on the 18 November 1987. An analysis of variance revealed no difference between days (not shown), so the data for the two days was pooled for subsequent analyses.

A paired t-test was performed on the difference between the proportions of visits to the two phases and the proportions of the two phases available on that plant. The null hypothesis that the flies were utilising each of the flower types in proportion to their abundance, i.e. that they

were not preferentially selecting one or other type over the other, was tested. The computer program STATISTICS (1988) was used for this analysis.

It was not possible to measure the minute amounts of nectar in the flowers because the floral tube and the quantity of nectar were too small. Hence it was not possible to determine whether post-presentation flowers produce nectar.

Period of stigma receptivity

To investigate the period of stigma receptivity, flowers of varying age were hand-pollinated in the field, and the seed set was subsequently measured. Flowers from four plants were assigned to four age classes:

1. Female - flowers open but anthers yet to dehisce,
2. Male - anthers dehiscing or dehisced and scales still bright yellow,
3. Fading - flowers just entering the post-presentation phase, with scales beginning to fade,
4. Faded - scales fully faded.

Pollen from a flower of another plant was brushed onto each recipient stigma, and the stigma was examined with a 20x hand-lens to ensure that it had ample pollen. The calyx was marked with a small dab of enamel model-maker's paint, the age of flowers being indicated with different coloured paints. After about four weeks, the nutlets had reached maturity and the number of nutlets per calyx was recorded.

An analysis of variance was performed on the number of seeds set per flower, with the parent plant and the age class of the flower as independent variables. The plant effect is a random one while the age class is a fixed effect, so the plant mean square was tested over the error means square and the class mean square over the interaction mean square (Sokal and Rohlf 1981). This analysis was performed with the GLM procedure of SAS (SAS Institute 1985).

Effective phase duration - female function

On 12 November 1987, a series of flowers was followed over the course of the day, and the time of pollination was assessed. For this trial, flowers that were just beginning to open were marked with tooth-picks in the morning. About 50 flowers were chosen each day. Each hour subsequently the stigmas were examined with a 20x hand-lens to check if pollen had been deposited on the stigma occurred. At the same time, the sexual stage of the flower was also recorded. It was not possible to count the number of pollen grains on the stigmas, but whether some pollen had been deposited or not was noted. Since there are four ovules per flower, only a small amount of pollen is required to set full seed. Moreover, the flowers are self-compatible (Chapter 1), so it is probable that once pollen is visible on the stigma, the flower has been adequately pollinated.

Each hour, the abundance of pollinators active on plants in a semi-circular plot of about 8 m in radius was also recorded. The total number of individuals of *P. huttoni* entering the plot over a period of 15 minutes was measured. This plot was adjacent to the area containing the plants with marked flowers.

Effective phase duration - pollen removal

The rate of pollen removal was measured on 22 November 1988 by counting the pollen remaining after a known period of time. This was compared with the amount of pollen found in flowers with intact anthers used for the pollen:ovule ratio estimate (Chapter 2).

Flowers were chosen at random at 9.00 a.m. just as they were opening and they were marked with toothpicks. In total, 80 flowers were marked and approximately 10 of these were removed at the following times:- 1.5, 2, 4, 7 hours after opening at 10.00 a.m. and 2.00 p.m. the next day and finally at 10.00 a.m. and 2.00 p.m. the third day. Because pollinator activity occurs only between approximately 9.00 a.m. and 6.00 p.m., the times of sampling were equivalent to the following periods of pollinator activity:- 1.5, 2, 4, 7, 10, 14, 19 and 23 hours after flower opening. As each flower was removed, its sexual stage, including the number of anthers

dehisced and the condition of the scales, was recorded. Each flower was placed in small glass vials containing FAA. In the laboratory, the amount of pollen remaining was determined using the whole flower method described in chapter 2 for determining pollen:ovule ratios.

The relative amount of pollen remaining was expressed as a proportion of the amount contained in flowers with intact anthers. An exponential decline in pollen remaining was fitted by taking the natural logarithms of the means at each time interval and fitting a line-of-best-fit to these means. It was necessary to first subtract a residual amount of pollen - 7500 grains, the amount that was left as a seemingly unremoveable fraction (see Figure 3). The line was forced through 1.0 - the point which corresponds to all pollen remaining at time 0.

This model of pollen removal was used to generate an expected frequency distribution of the amount of pollen remaining on various flowers after each time interval. For this expected distribution, the rate of visitation per flower was estimated to be 1.0 visit per hour (see Chapter 4). It was assumed that the mean number of visits would thus be equal to the time interval since opening and that the scatter around this mean would follow a Poisson distribution. Being visited during each time interval is a "rare" event, and therefore the expected number of visits should resemble a Poisson distribution (Sokal and Rohlf 1981). In order to convert the number of visits to the amount of pollen remaining, it is necessary to know the rate at which pollen is removed during each visit. This was estimated by the rate of the exponential decline. Once this estimate is made, the expected distribution of pollen remaining can be calculated for each time interval from the expected number of visits to each flower.

The effect of climatic conditions on phenology

The measurement of pollen deposition and presentation was repeated on two further days, 18 November 1987 and 21 November 1987. The three days were quite different in climatic conditions. On 12 November, the weather was fine and warm and pollinators were abundant. On 18 November, however, it was cold and windy but with periods of bright sun. Finally 21 November was overcast and cool. On both these last days, pollinators were relatively scarce.

On 13 November 1987 and on the morning of 14 November 1987, steady rain fell. In the late morning, the rain stopped and the sun came out. In order to determine the effect of rain on the phases of the flowers, two static samples were taken using the method described in chapter 2. One sample was shortly after the rain had stopped, and the other was several hours later in the afternoon. An additional class of flower was recognized this day. These flowers had bright yellow scales but had brown anthers that bore no pollen. It was apparent that these flowers had opened and began dehiscing their pollen before the rain and subsequently the pollen had been damaged. Though they were presenting no pollen, their scales had not faded, so they had not entered the post-presentation phase.

RESULTS

Phase Preferences of Pollinators

Individuals of the tachinid fly *Protohystericia huttoni*, clearly prefer to visit flowers that are in the male or female phase rather than those flowers that have entered the post-presentation phase (Table 1). Post-presentation flowers represent almost 50% of the flowers available, yet only about 15% of the visits are to these flowers ($t = 6.95$, $p < 0.0001$). The preference may be expressed as the following ratio,

$$\frac{\text{visits to receptive flowers}}{\text{visits to non-receptive flowers}} \Big/ \frac{\text{no. of receptive flowers}}{\text{no. of non-receptive flowers}}$$

in this case,

$$\frac{0.844}{0.156} \Big/ \frac{0.543}{0.457} = 4.55$$

Individual flies, therefore, display close to a five-fold preference for flowers in the presentation phase. Moreover, it was noticeable that the post-presentation flowers that were visited were generally those with scales that were just starting to fade and so represent the youngest of the post-presentation flowers.

Table 1. The frequency of visits to receptive and non-receptive flowers.

	Prop. of visits	Prop. of available flowers
Receptive	0.844	0.543
Non-receptive	0.156	0.457
Standard error (n=24)	0.039	0.039

Receptive flowers have bright yellow corolla scales, non-receptive flowers have fading or faded scales.

Table 2. The effect of the plant and the age class of the pollen-receiving flower on the success of hand pollinations.

	MEAN SEED SET ± S.E. (n)			
PLANT	FEMALE	MALE	FADING	POST-PRESENTATION
12	1.5 ± 1.50 (2)	4.0 (1)	2.2 ± 0.75 (6)	3.5 ± 0.40 (10)
117	4.0 (1)	3.0 ± 1.00 (2)	2.6 ± 0.75 (5)	2.6 ± 0.60 (8)
110	4.0 ± 0.00 (2)	2.7 ± 0.74 (7)	3.7 ± 0.33 (6)	2.8 ± 0.49 (8)
119	0.0 (1)	1.5 ± 1.50 (2)	0.0 ± 0.00 (3)	0.0 ± 0.00 (5)
AVERAGE	2.5 ± 0.81 (6)	2.7 ± 0.50 (12)	2.4 ± 0.39 (20)	2.5 ± 0.31 (31)

ANOVA

Source of variation	df	S.S.	F	P
Model		15	89.58	
Plant	3	49.09	7.77	0.0002
Age Class	3	2.74	0.36	0.7807
Plant * Age	9	22.61	1.19	0.3195
Error	68	201.25		

This is a mixed-model Anova, so the age class mean square was tested over the interaction mean square.

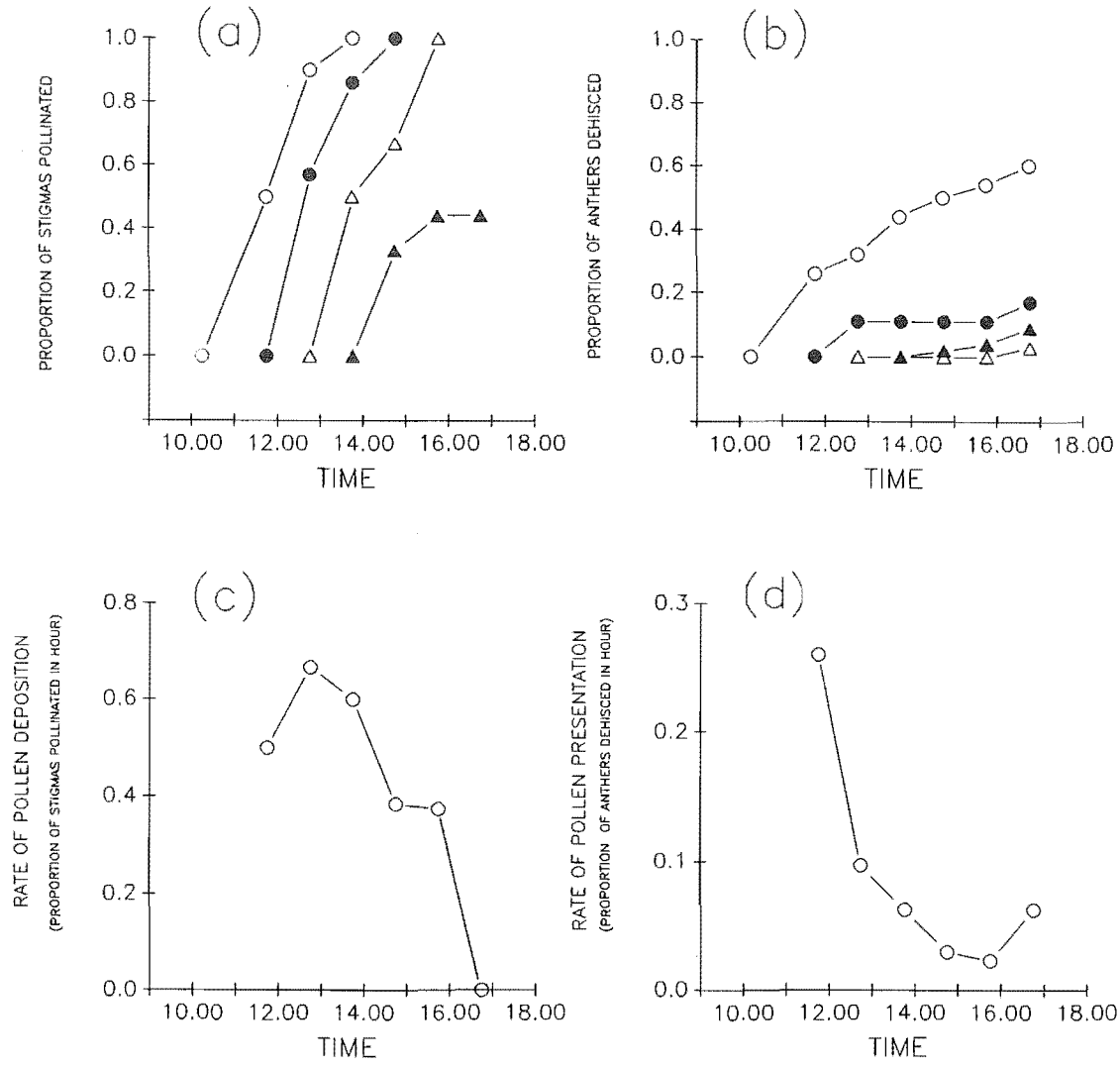


Figure 1. The fate of flowers from their opening on a fine and warm day (November 12 1987). The different samples represent the different opening times of the flowers.

a the proportion of flowers with pollen on their stigmas.

b the proportion of anthers dehisced.

c the hourly rates of pollen deposition.

d the hourly rate of anther dehiscence.

Period of stigma receptivity

Results of hand-pollinations of stigmas from flowers of different age show that the stigma remains receptive throughout the life of the flower (Table 2). An analysis of variance reveals that there are no significant differences in stigma receptivity throughout the flower lifetime.

Effective phase duration - female function

Figure 1 presents the results of the time of first pollination for flowers marked on 12 November. It is clear that pollination occurred quickly and was completed within a few hours (Figure 1(a)). With the exception of the flowers that opened after 1.00 p.m., all stigmas had received pollen by the end of the afternoon. Moreover, the rate of pollen deposition on previously unpollinated stigmas remained high throughout the day until about 5.00 p.m. (Figure 1(c)). This relatively high rate of deposition coincided with high rates of pollinator activity (Figure 2(a)). The onset of anther dehiscence is variable but generally has only just started or has yet to begin by the time pollination has occurred (Figure 1(b)). For only a small fraction of flowers were any anthers dehisced before pollination had occurred. This result was particularly evident during the afternoon, when anther dehiscence was particularly slow (Figure 1(d)). Table 3 presents a comparison between nominal and effective phase duration. The "half-life" of the female phase is given by interpolation of the curves from Figure 1. The period over which 50 % of the flowers had had pollen deposited was estimated for each morning run of flowers and averaged. Additionally the time for taken for half the flowers to dehisce one anther, and the time for all anthers was also estimated from Figure 1. Thus, it is possible to directly compare the accuracy of an estimate of the length of the female phase made from the timing of pollen release with the estimate derived from observations of pollen deposition. Considering for the moment only the fine and warm day (November 12 1987), the rate of pollen deposition exceeds the rate of anther dehiscence. On average, half the flowers are pollinated in just under one hour. In comparison, it takes approximately two hours for the first anther is dehisced on the average flower and over four hours for all anthers to dehisce. In this case, the effective female phase is shorter than the nominal duration assessed by the rate of anther dehiscence. The data for the two later dates will be considered below.

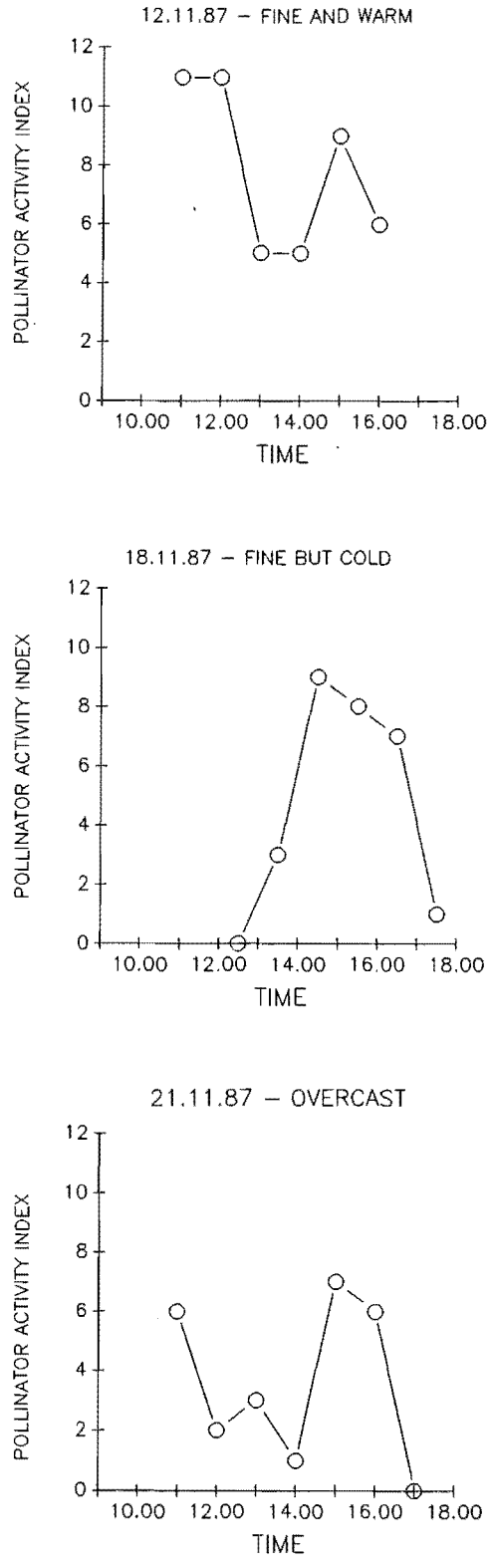


Figure 2. The rates of pollinator activity on the three days that the female function was surveyed. The index of pollinator activity is the number of individuals of *Protohystricia* observed foraging in a semicircular plot over a 15 minute period.

Effective phase duration - pollen removal

Figure 3 presents the results of the observations on the removal of pollen from marked flowers on a fine day. The results show a gradual removal of pollen down to a residual level. This residue is apparently not able to be removed, possibly because it has been dislodged to inaccessible regions of the flower or remains hidden in the anther sacs. The results reveal that pollen removal is a slow process and takes approximately 12 -14 hours of pollinator activity to reach the basal level. If we assume a rate of visitation of one visit per flower per hour (see Chapter 4), we can see that 12 - 14 visits are required to complete the male function. A model of exponential decline was fitted to the data, (see methods for details). The line of best fit was back-transformed and plotted on Figure 3(a). The relationship between the amount of pollen remaining and the time since opening is as follows:-

$$\text{Pollen Remaining} = \left(0.857^{\text{time}} + 7500\ (\text{Basal Level})\right) \times 35072\ (\text{initial amount})$$

This reveals that 14.3 % of the removeable pollen is removed each hour, which corresponds roughly to each visit.

Figure 3(b) shows the rate of pollen dehiscence and the rate of onset of the fading of corolla scales. It can be seen that the period of active pollen removal slightly exceeds the period over which anthers are dehisced. Throughout the first 8 hours of pollen removal, anthers are being dehisced and presenting new pollen to be removed. There is a short period of time in which no new anthers are dehisced, but pollen removal continues. It seems likely that the slow rate of anther dehiscence ensures that multiple visits are required to remove all the pollen. This is in contrast to the female function which seems to be satisfied within a few hours or visits on a fine day (Figure 1(a)).

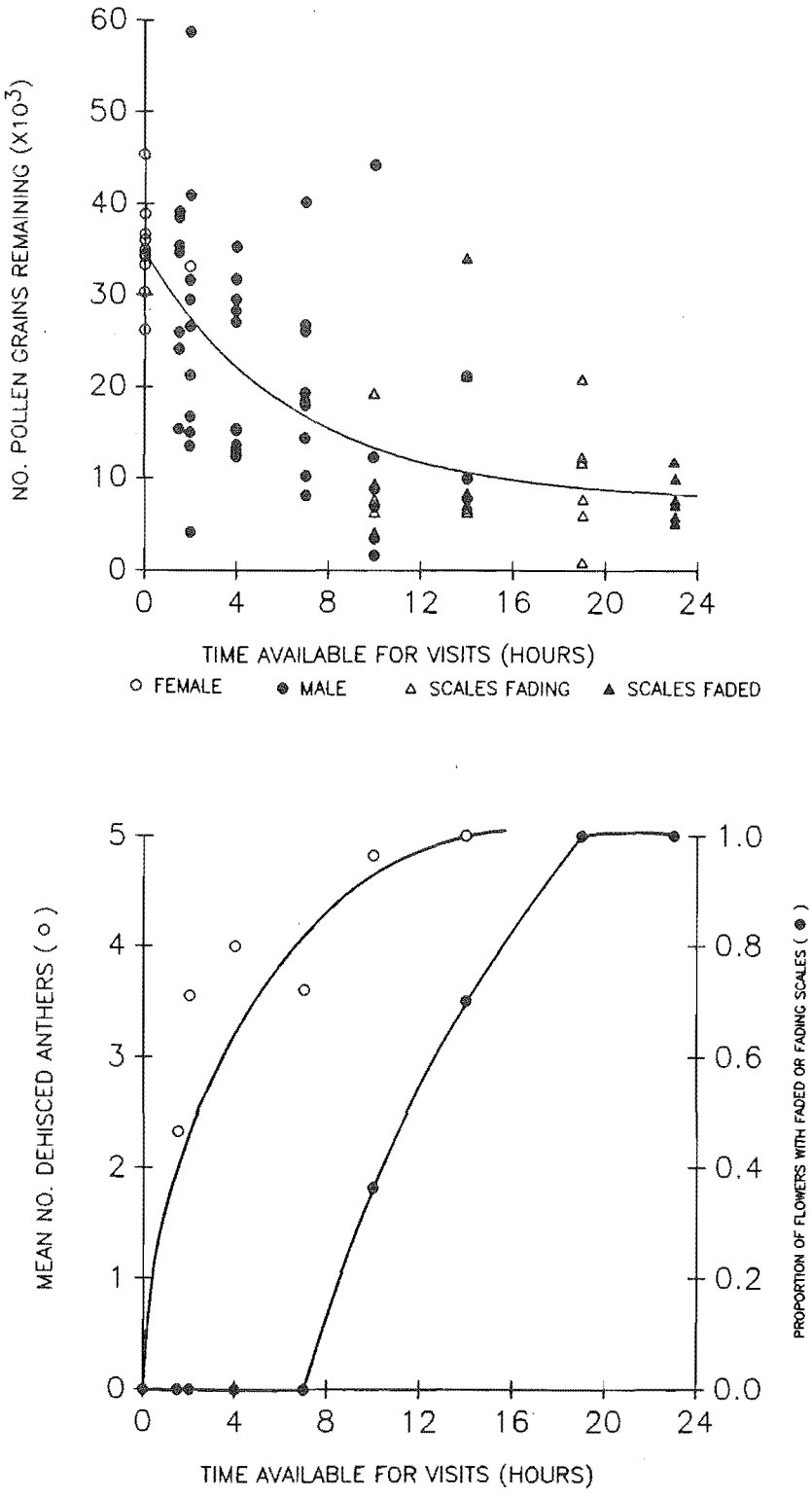


Figure 3. Pollen removal and phase development

a Removal of pollen from marked flowers over time. The symbols represent the phase the flowers were in at the time of sampling.

b The mean number of anthers dehisced and the proportion of flowers that have reached the post-presentation phase against time.

Table 3. The "half-life" of effective and nominal phases. The time taken for stigmas on half of the flowers to be pollinated (effective female phase duration) was interpolated from Figures 1, 6 and 7, and averaged across the day. The time taken for half the flowers to dehisce one of their anthers and to dehisce all 5 anthers.

DATE	EFFECTIVE FEMALE PHASE DURATION (HOURS)	NOMINAL FEMALE PHASE ONE ANTHER	NOMINAL FEMALE PHASE ALL ANTHERS
12.11.87 Fine and Warm	0.9	2.0	4.3
18.11.87 Fine but Cold	5.1	1.3	6.1
21.11.87 Overcast	5.0	1.5	8.6

Only flowers that opened before 2 pm were used for the interpolations above.

Table 4. The "half-life" of effective and nominal male phases. Shown is the time taken for each half the pollen to be removed and for half the flowers to enter the post-presentation phase, interpolated from Figure 4.

DATE	EFFECTIVE MALE PHASE DURATION (HOURS)	NOMINAL MALE PHASE DURATION (HOURS)
22.11.88 Fine and Warm	4.0	11.5

A similar comparison to that made above between nominal and effective phases for the male function. In this case, the time taken for half the pollen to be removed is compared the time taken for half the flowers to enter the post-presentation phase. The point at which 50% of the flowers have reached either the post-presentation phase or had half their pollen removed is interpolated from Figure 4 as before. The results (Table 4) reveal that the average flower has half of its pollen removed after approximately four hours. It takes almost twice as long again for half

the flowers to enter the post-presentation phase. However, because of the exponentially declining rate of pollen removal, a considerable amount of additional time is required to remove the remaining fraction of pollen that is eventually removed. As a result, pollen removal continues for as long as 16 hours after flower-opening. In this sense, there is good correspondence between when pollen ceases being removed and the onset of the post-presentation phase. The result of the phase-preference study is supported by the rate of removal, as there is no further decline in pollen remaining in flowers after the phase change.

Figure 3(a) also reveals that there is a good deal of variation in the amount of pollen remaining on a flower at a given time interval. In particular, while individual flowers are sometimes quickly relieved of their pollen, there are often flowers that apparently have been missed and still retain most or all of their pollen even after a considerable amount of pollinator activity time has elapsed. An attempt to model this situation is presented in Figure 4. Figure 4(a) shows the expected range and frequency of remaining pollen, assuming that the calculated rate of removal of pollen (14.3% per hour) applies. It is also assumed that the rate of visitation follows a Poisson distribution - that is, that the chance of visitation is independent of the number of visits previously made and that the mean number of visits is much smaller than the theoretical maximum number of visits that is possible. Both assumptions seem reasonable on biological grounds. Because multiple visits remove a successively smaller amount of pollen, the expected amount remaining becomes compressed near zero as the time interval increases (Figure 4(a)). Additionally, the number of flowers at the top end of the distribution i.e. those that have experienced few or no visits, should become successively rarer as time goes on. The expected variance increases to a maximum toward 7 hours (Figure 5) and decline again.

Figure 4(b) displays the actual observed distributions of remaining pollen at a series of times after flower opening. For clarity, the result for 1.5 hours and 2 hours have been pooled. Also shown is the distribution of pollen of flowers before anther dehiscence to indicate the amount of variation likely to be encountered either as a result of sampling error or as an effect of differences between flowers in pollen production.

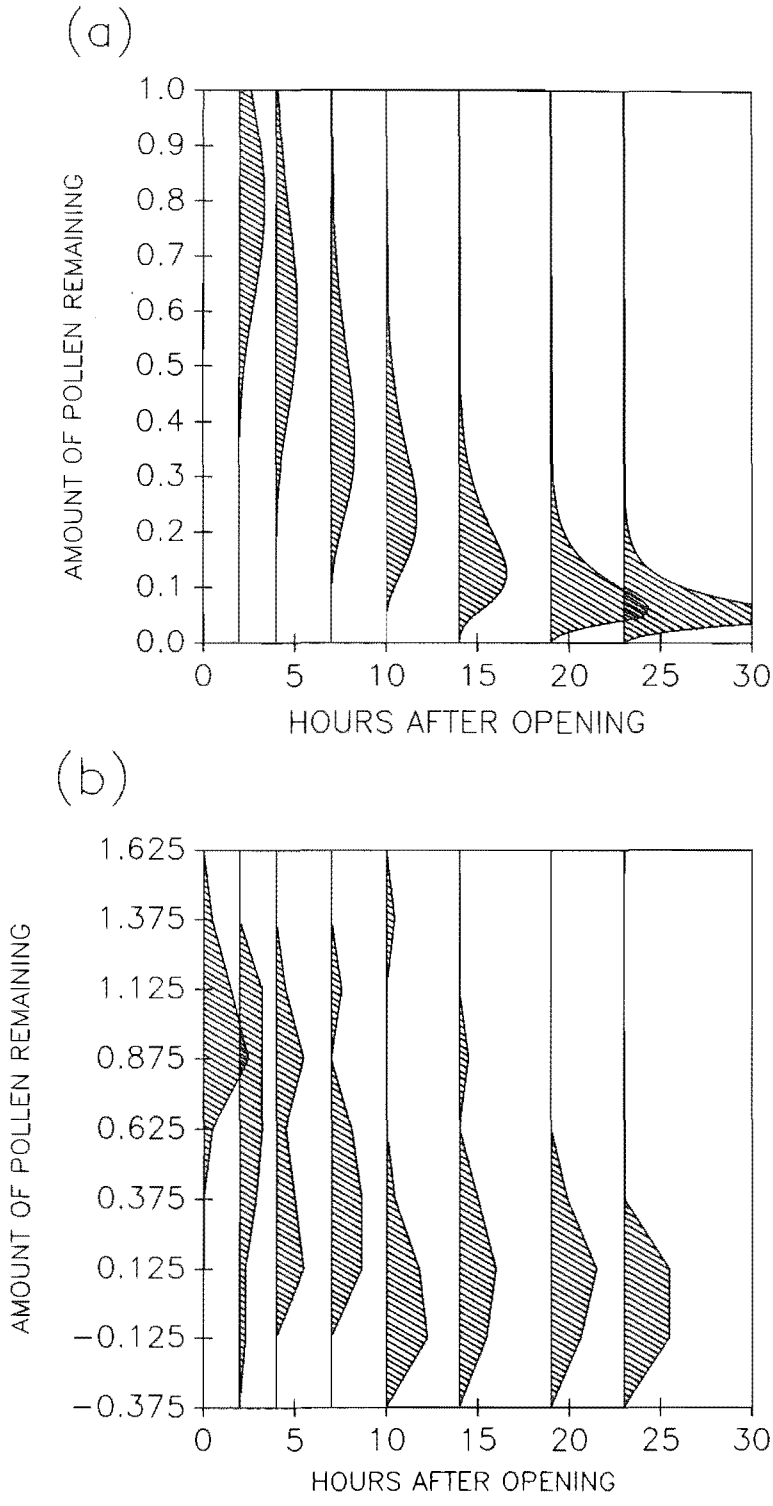


Figure 4. The expected,(**a**) and observed, (**b**) distribution of flowers with varying pollen remaining against time. See text for derivation of expected distributions. The vertical axis in (**b**) exceeds the limits of 1 and 0 because of the variation between flowers in the amount of pollen they contain initially and how far below the average basal level the remaining pollen falls. (See text for explanation of this basal level).

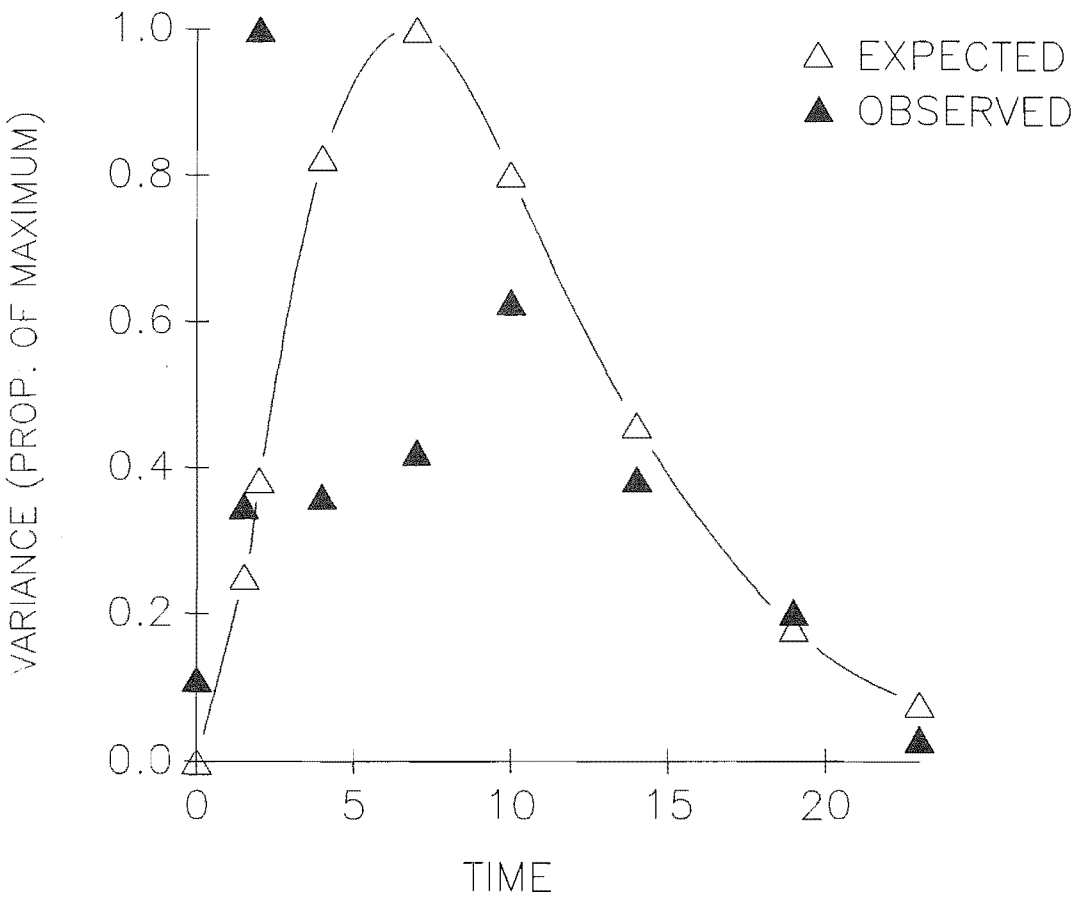


Figure 5. The expected and observed variances of pollen remaining against time. See text for derivation of expected variance.

This variation will occur on top of the variation expected from visitation history of a particular flower. The general coincidence of the means between Figures 4(a) and (b) is expected, as the shape of the decline curve for Figure 4(a) is based on the data presented in Figure 4(b). It can be seen however, that the variance increases and then declines as time passes as expected form Figure 4(a) (Figure 5). The general agreement between the observed and expected distributions agrees with the assumption that pollination follows a Poisson situation and that it can reasonably be expected that the mean pollen removal will decline moderately quickly at first but that it may take some time for the tail of the distribution to reach a low level. The observed interval of about 12 hours, in this case at least, is sufficient to allow these flowers in the tail of the distribution to also be visited often enough to satisfy their male function.

The effect of climatic conditions on phenology

The importance of the weather at the time of flower opening is revealed by the comparison of Figures 6 and 7 with Figure 1. Table 3 reveals the comparison between nominal and effective female phase durations. On the two cooler days, the result is somewhat reversed on the fine and warm day. A much longer time is required for half the flowers to be pollinated. However, the rate of pollen presentation remains as fast or faster than on the fine and warm day. The relatively poor weather on both of the later dates led to slower pollen deposition. On 18 November, only about 60% of the flowers that had opened in the morning had been pollinated by the end of the day (Figure 6(a)). Throughout the day, pollination was relatively slow (Figure 6(c)) despite a moderate numbers of pollinators in the afternoon (Figure 2). However, anther dehiscence is as fast or faster than on the fine day (Figure 6(d)). The timing of anther dehiscence seems to be complex; it may involve an interaction between temperature, light and the time of day. On a fine day, anthers appear to dehisce quickly in flowers that open in the morning but much more slowly in those that open in the afternoon (Figures 1(b), (d)). However, on the cold day (18 November), dehiscence occurred slowly in the morning and increased during the day (Figures 6(b), (d)). On the overcast day (November 21), dehiscence was fairly constant throughout the day (Figures 7(b), (d)). Pollinator activity is intermediate during the day (Figure 2) and this is reflected by an intermediate rate of pollen deposition (Figure 7(a), (c)).

The rate of pollination should be correlated with pollinator activity, if the method of censusing pollinator activity is accurate. The relationship between the hourly rate of pollen deposition and pollinator activity is shown in Figure 8. Although the correlation is not quite significant ($r = 0.4314$ $p = 0.0738$), this lack of fit is affected by a handful of outliers. For example, there are quite a number of pollinators around at 11.00 a.m. on the 21st but no pollination. This is probably due to the habit of *Protohystricia* of spending this time of the day on reconnaissance flights without actually visiting flowers (see Chapter 6). Another possible cause of the lack of fit is the different slopes of the relationship between activity and deposition on the different days. In particular, on fine days, more pollination occurs for the same amount of activity than on a less favourable day.

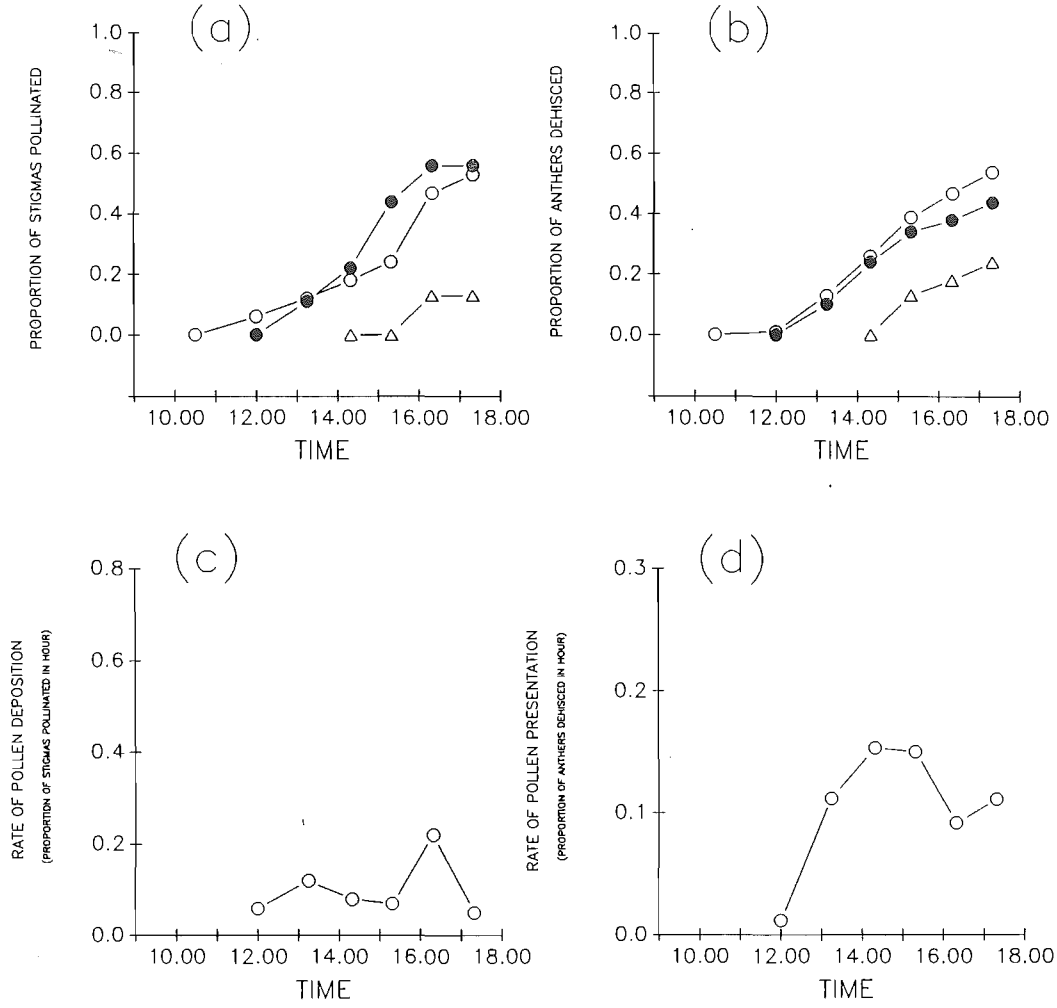


Figure 6. The fate of flowers from their opening on a fine but cold day (November 18 1987).
a the proportion of flowers with pollen on their stigmas.
b the proportion of anthers dehisced.
c the hourly rates of pollen deposition.
d the hourly rate of anther dehiscence.

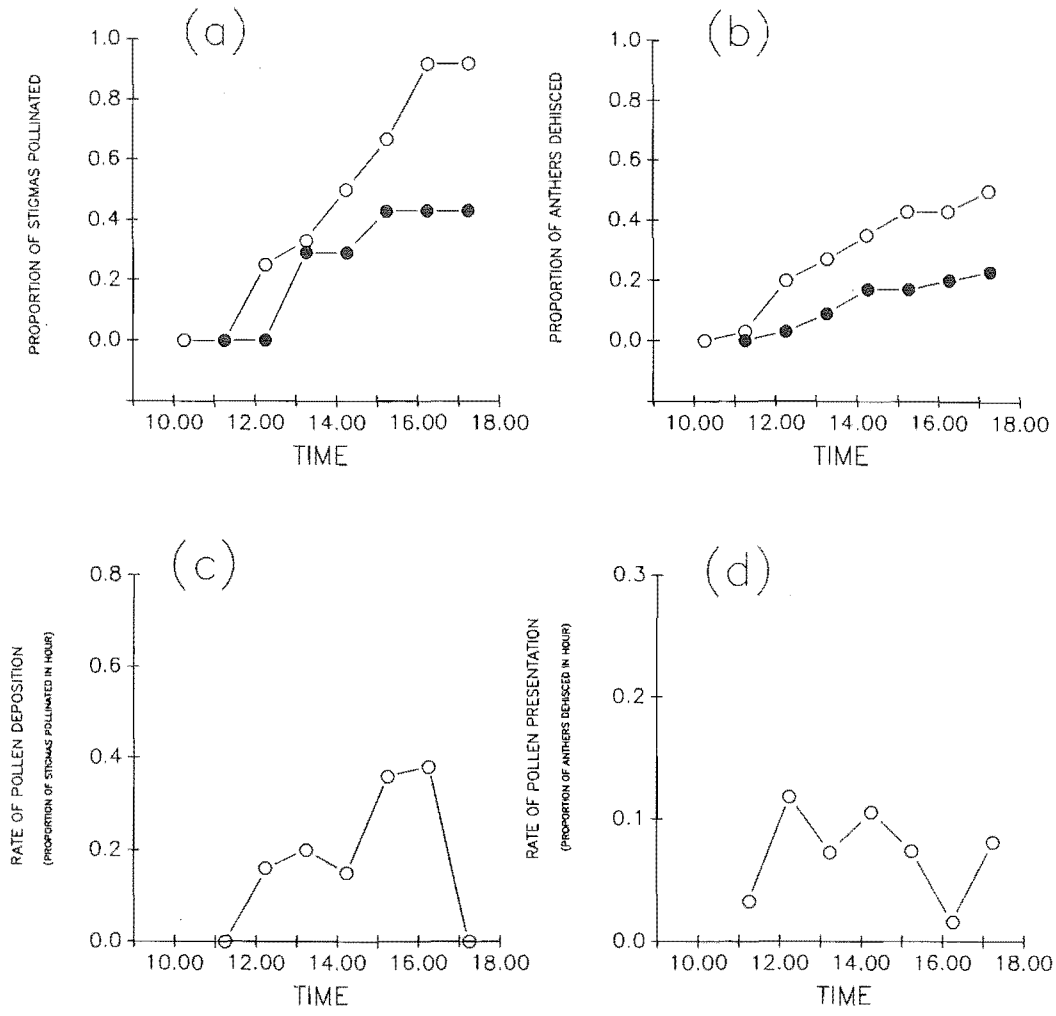


Figure 7. The fate of flowers from their opening on an overcast day (November 21 1987).
a the proportion of flowers with pollen on their stigmas.
b the proportion of anthers dehisced.
c the hourly rates of pollen deposition.
d the hourly rate of anther dehiscence.

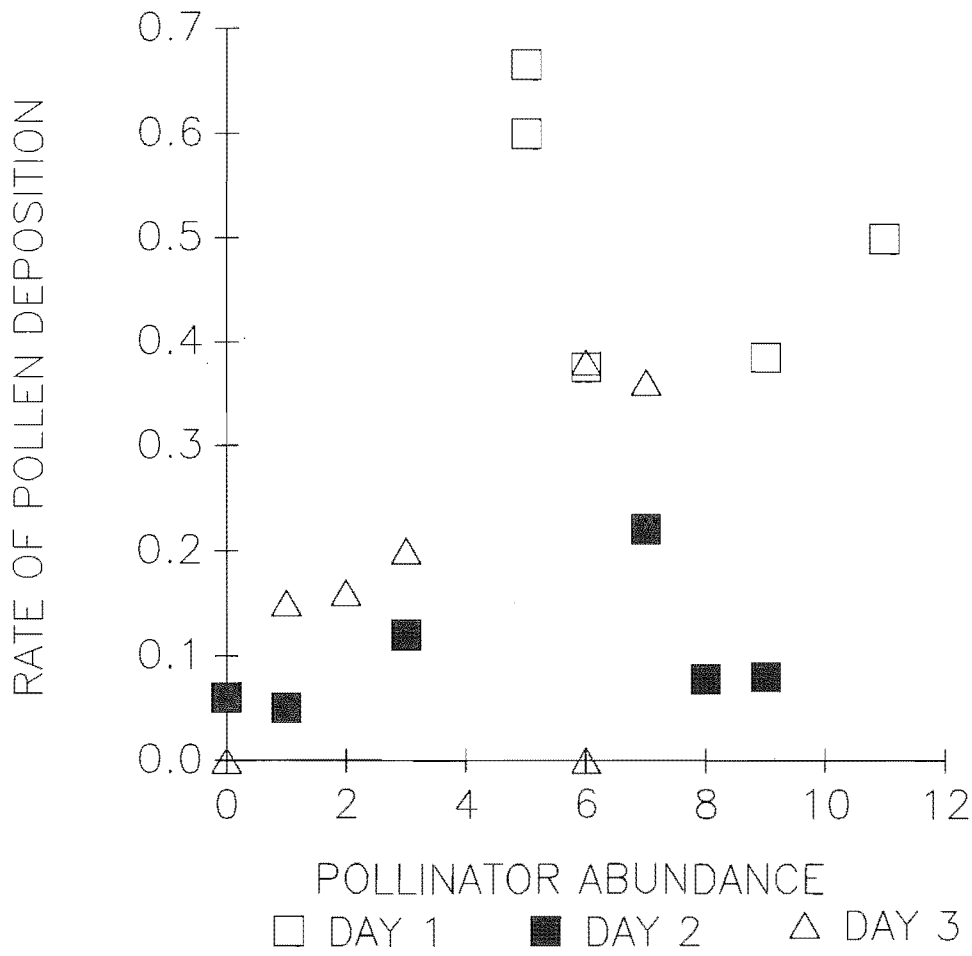


Figure 8. The relationship between the rate of pollen deposition and pollinator activity in the sample plot for each of the three sampling days.

These differences are suggested by the location of the points on the scatter diagram for the different days.

The importance of weather on the phenology of flowers is confirmed by the effect of overnight rain on the proportion of flowers in the various age classes (Figure 9). Prior to the rain, there was the usual balance of flower classes, with a small number of female-phase flowers and an approximately equal number of male- and post-presentation-phase flowers. After the rain, however, there was an increase in the number of female-phase flowers, presumably because the onset of anther dehisence was delayed. In addition, a number of flowers that were presenting pollen the previous day were rendered non-functional by the rain. The pollen had been lost from these flowers despite their having bright corolla scales.

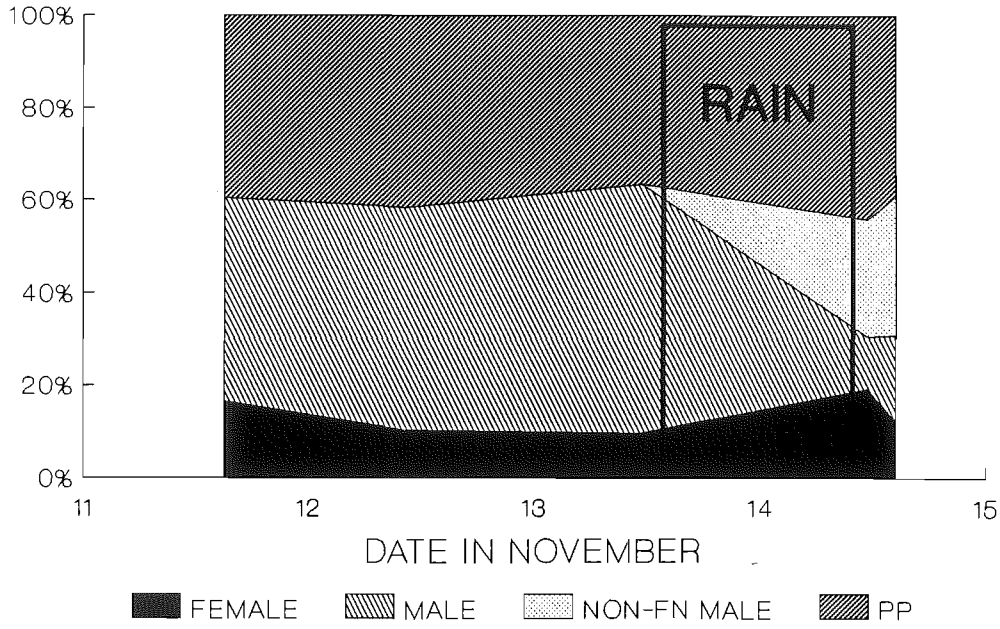


Figure 9. The effect of overnight rain on the proportion of flowers in four recognized age classes. (Non-fn male = flowers that have damaged anthers but yellow scales, PP = post-presentation phase flowers).

The net effect of these changes was a swing in the balance of phases in favour of the female stage and a loss of pollen-presenting flowers. Thus, the female function of flowers that were opening or opened during this time was maintained, while the male function suffered from the unavoidable loss of pollen and opportunities for visitation. Once the period of bad weather was over, the flowers whose dehiscence was delayed, quickly presented their pollen and the balance of phases was swiftly restored.

DISCUSSION

Ideas on sexual selection in plants have suggested that many of the adaptations of hermaphrodite flowers are concerned with the dispersal of pollen rather than its receipt. It is expected that in addition to allocating more resources to the male function at anthesis, there will be selection for prolonged pollen presentation (Willson 1979, Lloyd and Yates 1982, Queller 1983). Although the relative duration of pistillate and staminate phases has often been given in studies of the reproductive biology of species, generally these results are in terms of the physical appearance of anthers and stigmas. Alternatively the period of stigma receptivity has been measured. However, the time over which pollen is actually available for removal to other flowers or is able to be deposited to stigmas with a chance of successful fertilisation is likely to be shorter than the nominal phases indicated by previous studies (Lloyd and Webb 1986). For example, Richardson and Stephenson (1979) using a visual assessment of phase duration state that the pistillate phase is actually longer than the staminate phase in contrast to the expected result. However, if sufficient pollen is deposited onto stigmas quickly enough, and pollen deposited after this has no chance of fertilisation, the female phase may be effectively over. To attribute the remaining period in this protandrous species to the female function would be erroneous. The period over which pollen can be successfully deposited was not given. Palmer et al (1989) suggested that pollen of *Amianthum* was presented for two days while the "pistils maintain a high function for 6 days" based on the period that stigmas can be pollinated if unvisited. Devlin and Stephenson (1985) did measure the pollen production in *Lobelia cardinalis* by removing the pollen available on successive days but estimated the duration of the pistillate phase by the timing of stigma presentation. There are very few studies that measure both pollen availability and time of pollen receipt. Webb and Bawa (1983) working with *Malvaviscus arboreus* found that both functions are satisfied early in these one-day flowers. I would assert, however, that pollen receipt is likely to be satisfied first (cf Bell 1985), as there are 5 ovules (Hutchinson 1967) and enough pollen grains to fertilise this number are deposited before all of the pollen is removed. Similarly, Wolfe and Barrett (1989) found that flowers of the tristylous *Pontedaria cordata* are visited between 10-20 times per day, and pollen is able to be removed throughout the day. In

contrast, a single visit deposits, on average, 14-53 compatible grains on a stigma. There is only one ovule in this species, so a single visit is easily able to satisfy the female function. Thus in these cases there is evidence that it is the male function that is being emphasised at anthesis.

Data collected here for *Myosotis colensoi* show that nominally, stigmas may be pollinated successfully throughout the life of a flower. Similarly, results (unpublished) show that pollen from the very oldest flowers are still able to successfully father seeds. It would appear that like *Amianthum*, the female phase duration exceeds the male function. However, stigmas usually have pollen deposited within a few hours of opening. It is likely that these first grains will successfully fertilise the four available ovules. A comparison of the effective female duration (the period when pollination is possible) with the time taken for anthers to dehisce, reveals that the nominal female phase (as measured by the onset of the male phase) takes approximately twice as long as the effective female phase. The total period of stigma receptivity is therefore a very poor measure of the effective female phase and the nominal phase length based on anther dehiscence is also approximate.

In addition, pollinators are reluctant to visit flowers that have fading or faded scales. This result is confirmed by the completion of pollen removal by the time of the phase change. Clearly, therefore, these flowers spend a long time in a phase in which they make no direct contribution to pollen donation or receipt. The adaptive significance of maintaining such non-productive flowers will be considered in the next chapter. For the purposes of this chapter it is necessary to note only that the period of post-presentation cannot be assigned directly to either male or female functions; their function, if any, must be accessory.

In short, on a good day, by approximately the time anthers dehisce, the stigmas have already been successfully pollinated. Then follows an extended period of pollen donation which is followed in turn by a period in which neither pollen donation or receipt is possible. The results indicate that as many as 12-14 visits may be received before all pollen is removed. The prediction of a relatively longer male function is clearly supported.

The five anthers do not dehisce their pollen simultaneously but do so over most of the period of pollen presentation. Such packaging is likely to extend the number of visits that are able to effectively disperse pollen. Previous studies have shown that visitors frequently remove most of the pollen accessible in just one visit (Strickler 1979, Harder et al 1985, Harder and Thomson 1989, Wolfe and Barrett 1989), although in all these cases pollen is the main reward for pollinators rather than nectar. Hence, a flower that presents all its pollen at once is likely to have its pollen transported by fewer pollinators, and thus to fewer potential mates, than one that staggers its release. The gradual release of pollen will confer advantage to the male function as long as visits are relatively common and certain (Lloyd and Yates 1982, Wells 1988, Harder and Thomson 1989).

While the sequential dehiscence of pollen allows a relatively longer period over which pollen is removed, it also allows a greater assurance that the flower will be eventually visited during pollen presentation. The rate of one visit per hour is an average rate in fine weather in the middle of the flowering season. Some flowers will of course have to wait longer than this before the first visit occurs. Assuming the chance of being visited remains equal throughout the presentation phase, the number of visits flowers receive should follow a Poisson distribution. Expected frequency distributions based on the observed removal rate and visits following a Poisson distribution around the mean of one visit per hour reveal that the variance of pollen remaining in flowers remains high until 7 hours have elapsed. Until this time there remains a fairly high probability that some flowers will not have been visited. Anther dehiscence stops after about 8 hours, so that visits after this time may remove all the pollen. If visits occur before this time, only as much pollen as has been released from anthers is available. Thus, the relatively slow dehiscence and delayed scale colour change allow both the number of visitors exporting pollen and the certainty of being visited to be maximised.

Until now we have considered pollen deposition and removal in periods of optimum weather. This is typical of most studies of pollination. However, it seems likely that on days on which pollinator activity is restricted, the flowers that open will in general face a very different fate

from those that open under better circumstances. Despite this, few studies have addressed this aspect directly. Wells (1988) found significant correlations between the time taken for anthers to dehisce in *Pseudowintera colorata* and ambient temperature and that the weather pattern imparted a loose synchrony of plants within a population. Similar temporal synchrony has been found in *Aralia* (Thomson and Barrett 1981) and Lobelia (Devlin and Stephenson 1987). Neither of the latter studies attempted to relate the differences observed to the weather. Such fluctuations in gender are likely to impart quite important influences on the success as male or female parents. In particular, male-male competition will be less intense when phenotypic gender swings to towards the female side (usually in periods of bad weather). In addition male fitness of a flower will be increased if its pollen can be protected or conserved in periods of bad weather (Percival 1955) and yet made available as soon as conditions allow transport to receptive stigmas (for an early expression of this argument see Kerner 1902). Pollinator activity is known to be dependent on weather, particularly temperature (Telas 1976, Schemske 1977, Ramsey 1988). Thus, rates of pollen turnover are expected to depend, in part, on weather. Accordingly, Webb and Bawa (1985) found significantly lower pollen loads on stigmas on days which were damp and overcast or very windy.

The above discussion relates to those species where phenology is influenced by the weather. When the proximate cues for flower development and pollinator activity are similar, there may be a synchrony between the two and the integrity of pollen transport thus maintained. A similar result occurs in those species whose phase durations are induced directly by pollinator activity. In a handful of species, the rate of pollen removal and deposition has been shown to affect the lengths of the staminate and/or pistillate phases (Devlin and Stephenson 1984, Webb and Littleton 1987, Richardson and Stephenson 1989).

In *M.colensoi*, periods of rain delay the dehiscence of anthers. This protects flowers from losing pollen but also causes a change in the proportions of male and female flowers. During rain, flowers continue to open but do not dehisce their anthers. Once rain stops, therefore, there is an over-representation of female flowers and a flower that enters the male phase will enjoy

period of relatively lax competition for stigmas. There may be selection for rapid presentation of the pollen as soon as conditions become favourable. This may explain why flowers continue to open during rain.

While there is a strong effect of rain on flower development, the relationship between weather conditions, pollinator activity and pollen presentation on days without rain is less clear. It appears that the rate of pollen deposition is slower on less favourable days and that this rate depends on pollinator activity. Comparing the effective timing of the female function on the cooler days reveals that there may be an extensive overlap between the male and female functions. Many stigmas have not been pollinated before the anthers begin to dehisce on a flower. Autogamous selfing is therefore more likely should an insect visit the flower. In situations where bad weather limits female success through pollinator limitation, the maintenance of stigma receptivity may allow a more assured reproductive success. This argument is parallel to the situation discussed in assessing the adaptive significance of a delayed-selfing strategy.

The effect of variation in weather on pollen removal was not assessed and the rate of presentation of pollen shows no clear relationship with ambient conditions. Even if pollen presentation is not closely tied to pollinator activity, it is unlikely that either male or female function will fail. If visitation rates are lower on bad days, it is still likely that stigmas will receive visits sometime during flower opening and allow full seed set. In addition, when visits are low it is probably advantageous to present all pollen simultaneously under the supposition that flowers may receive only this one chance of exporting pollen on poorer days. It would be useful to know how much pollen is removed at a single visit and whether this proportion changes as the amount of pollen presented is altered. If the proportion is high and remains so even when all pollen is presented, a mechanism of pollen packaging and gradual release rather than a pollen dispensing strategy may have advantages when pollinator activity fluctuates. Thus, it may be advantageous to maintain rates of pollen presentation because of rather than in spite of low visitation rates. This reasoning may also explain the restriction of pollen dispensing mechanisms to a handful of species which receive a high and reliable rate of visitation. Pollen packaging, on the other hand,

may be very common (Percival 1955) because of the flexibility in dependence on high visitation rates.

CHAPTER 4

THE EFFECT OF FLOWER DISPLAY SIZE ON VISITATION RATES AND REPRODUCTIVE SUCCESS IN *M.COLENSOI*

In 1948, Bateman made the observation that in both animals and oogamous plants, male fitness is usually more limited by access to mates while female fitness is usually more limited by resources available to mature her progeny (Bateman 1948). Rather belatedly, there has been a growing realization amongst botanists in recent years that the two functions in hermaphroditic plants should be treated separately, as following Bateman, the two components may have very different requirements of floral function. Appropriate measures of male and female fitness to measure the reproductive success of a particular flowering strategy should consider pollen removal and deposition and seed production on a per flower basis. Measuring reproduction this way reveals the efficiency of a strategy for either male or female fitness.

Previously, studies had emphasised the importance of selection for traits that ensure cross-fertilisation, and the success of adequate pollen receipt; in other words, selection on the female component of plant fitness. Evidence collected in recent times, however, has suggested that the male component of plant fitness may be more important in selection for secondary floral features (Willson 1979, Queller 1983, Sutherland and Delph 1984, Bell 1985, Stanton et al. 1986). In the light of this, recent studies on the importance of large floral displays have emphasised two main areas of interest. Work on the two families Orchidaceae and Asclepiadaceae (Willson and Rathcke 1974, Willson and Price 1977, Chaplin and Walker 1982, Queller 1983, Piper and Waite 1988), which present their pollen in aggregations known as pollinia, have led to the general observation that there many hermaphrodite flowers set no seed. These authors suggested that the "surplus" flowers are selected primarily for their role in increasing male fitness by increasing the potential number of mates their pollen reaches. Other adaptive functions of these surplus flowers have been postulated however that involve female fitness. These include the selective abortion of flowers either as a mechanism to regulate the number of offspring (Stephenson 1980,

Willson and Price 1980, Wyatt 1981, Bawa and Webb 1984, Bookman 1984) or to select the parentage of the successful embryos (Bertin 1982, Bookman 1984). Also, the potential surplus may allow reproductive assurance if predation of other flowers occurs (Lee and Bazzaz 1982, Bawa and Webb 1984) or resources for seed maturation are unpredictable (Lloyd 1980).

Meanwhile, others have suggested that the aggregation of flowers into large displays functions to increase the attractiveness of a plant and give it an advantage over smaller plants in competition for pollinators (Willson and Price 1977, Schaffer and Schaffer 1979). It is implied that a flower will do better as part of a large display than it would as part of a small one. This hypothesis depends on the rate of pollinator-visitation on a per flower basis being higher (there is an increased efficiency of pollination in plants with a larger floral display). That pollinators visit larger plants more often has been frequently demonstrated (Willson and Price 1977, Willson and Bertin 1979, Schemske 1980b, Davis 1981, Paton 1982, Thomson et al 1982, Bell 1985, Geber 1985, Andersson 1988, Cruzan et al. 1988). Similarly, rates of seed production *per plant* clearly increase with increasing plant size (Willson et al. 1979, Schemske 1980a, Udovic 1981, Pyke 1981a, Stephenson 1982, Wyatt 1982, Firmage and Cole 1988). Fecundity may not be a good measure of success in terms of attracting pollinators, however, as seed and fruit set is frequently more limited by resources than by pollination (Stephenson 1981). Total pollen removal also increases with an increase in flower number (Lynch 1977, Schemske 1980a, Chaplin and Walker 1982, Bell 1985, Wolfe 1987, Firmage and Cole 1988, Piper and Waite 1988). But the important parameters to consider the efficiency of processes are those that look at success per flower. Few studies have looked at reproductive success on a *per flower* basis, however. Visitation rates per flower did increase in some cases (Paton 1982, Klinkhamer et al. In press) but not in others (Schemske 1980b, Geber 1985, Andersson 1988, Bullock et al. 1989). Evidence on pollen removal or deposition per flower comes again mainly from pollinia-bearing species and is also inconsistent. Frequently, no significant effect of inflorescence size on per flower success has been found except for very small displays (Bell 1985, Piper and Waite 1988). Studies on the pollen transfer of species with free pollen are few and have sometimes shown increased removal or deposition per flower (Cruzan et al. 1988) and sometimes not (Geber 1985, Campbell 1989).

There is a clear need to investigate the effects of floral display size on aspects of reproductive success on species with free pollen (Bertin 1988). Pollinia-bearing species are a very specialised group of plants and the nature of the pollen transfer process may have aspects different from transfer in species with free pollen. It would also be desirable to conduct studies that determine whether pollination success increases, remains level or even declines on a per flower basis.

The persistence of flowers that are functionally non-reproductive, signalled by some sort of floral colour change has been demonstrated several times in unrelated taxa (see review in (Gori 1983). Explanations of the adaptive significance of these flowers usually presume that their presence increases the long-distance attractiveness of the display. At short distances they are assumed to direct pollinators away from functionally non-reproductive flowers (Schemske 1980b, Gori 1983, Lamont 1985). For this hypothesis to hold, it must be shown that these flowers are not visited directly and that their presence increases the rate of visitation to the remaining functional flowers. Few attempts to directly test these ideas have been made (Casper and La Pine 1984, Delph and Lively 1985, 1989, Gori 1989) and are inconsistent in their findings. An attractive function was demonstrated by Cruzan *et al* (1988) and by Gori (1989), but not by Casper and La Pine (1984) or by Delph and Lively (1989).

The aims of this chapter are (1) to evaluate the effect of floral display size on flower visitation rates and three parameters of reproductive success (pollen removal, pollen deposition and seed set); (2) to test the male-function and pollinator-attractiveness hypothesis for the selection of large floral displays and (3) to examine the adaptive significance of post-presentation flowers.

METHODS

Insect visitation rates and floral display

On three days in November 1988, a set of 32 adjacent plants of *M.colensoi* were observed for several hours each day. For each plant, the number of times an individual of *Protohystricia huttoni* landed and began foraging on the plant was recorded. I will refer to such

visits as bouts rather than visits to distinguish them from individual flower-visits. flower-visit may be simply be an "encounter" - where a flower is approached and may or may not result in a "probe". A probe refers to the act of inserting the proboscis into the floral tube. The data from the three days could not be pooled without "pseudoreplication" because the same plants were used each day. Instead, three separate regressions of display size (the total number of flowers presented regardless of phase) and number of bouts per hour were carried out. The number of flowers per plant was first log-transformed to obtain a more normal distribution.

On the afternoon of 11 November 1987, individual flies of *P.huttoni* were observed foraging on a group of *M.colensoi* plants. As a fly visited a plant, the number of probes made on a single plant was recorded. Subsequently, the number of presentation-phase flowers (with yellow corolla scales) and post-presentation phase flowers (with faded scales) was also recorded. Correlations and regressions between the floral display and the number of probes were made for all combinations of floral display (number of presentation-phase flowers and post-presentation flowers) and visitation rate. The number of probes made per presentation-phase flower was estimated by assuming that 84.4% of probes are made to presentation-phase flowers (see Chapter 3). This adjusted number of probes was divided by the number of presentation-phase flowers on the plant to obtain the rate of probes per flower.

To estimate the number of probes an individual flower in the presentation-phase state would receive in an hour, the previous two estimates were multiplied. The product of the estimated number of bouts for a given display size per hour and the number of probes made per presentation-phase flower at a single bout was found.

Reproductive success and floral display size

To assess the relationship between floral display and reproductive success three parameters of reproductive success were measured for plants varying in the number of flowers presented.

On 5 December 1988, one randomly chosen flower was removed from plants of different sizes just as the flower was entering the post-presentation phase. The flowers were placed in small glass vials containing FAA. The total number of flowers on each plant was counted. Subsequently, in the laboratory, the number of pollen grains on the stigmas was counted. The style was removed from the flower, softened for several minutes in heated 5% NaOH and stained in Calberla's stain. Pollen grains contrasted strongly with the tissues of the stigma and could easily be counted.

The number of pollen grains remaining on the flower was measured using the whole flower method for estimating the pollen-ovule ratio that was described in Chapter 2.

On 4 January 1989, nutlets were collected from a series of plants marked during the flowering period, six weeks previously. The number of mature nutlets per fruit was counted.

RESULTS

Insect visitation rates and floral display

On each of the three days surveyed, flies of *P.huttoni* made more bouts to larger plants than to smaller ones. The response was not linear, however, and the best line of fit was obtained with a log-log plot (Figure 1). The correlations for each day are all highly significant (Table 1) and the slopes of the regression lines similar in each case. There is a good relationship between display size and flowers probed, apart from three instances when only a single flower was visited (Figure 2(a)). The slope of the line is clearly less than one, showing that as the display gets larger an increasingly smaller proportion of the flowers are visited.

Chapter 3 showed that *P.huttoni* makes 84.4% of its probes to presentation-phase flowers. Taking this figure into consideration, it is possible to estimate the number of probes made to each presentation-phase flower in a single bout (Figure 2(b)). The number of probes declines from a figure greater than 1.0 probe per presentation-phase flower in small plants to

about 0.5 in large plants at each bout. The relationship is again highly significant (Table 1).

By combining the estimates of the relationships between display size and both the number of bouts per plant per hour and the number of flowers visited in a single bout, it is possible to estimate the number of times a presentation-phase flower will be visited each hour on plants of differing sizes (Figure 3). A different curve is shown for each of the three days. There is only a slight effect of plant size on visitation rates. It is estimated that each flower on small plants (with a total display of less than about 25 flowers), will receive less visits than flowers on larger plants. Above this plant size, there is only a slight increase in visitation rate. Because each of the lines is a product is the product of two other regression lines, it is not possible to test whether the slope is significantly different from zero. The curve probably has rather large confidence intervals and the true relationship could lie somewhere within a fairly large confidence envelope. However, it seems reasonable to expect that the average flower on average sized plant would be visited about once an hour.

Reproductive success and plant display size

Figure 4 shows the effect of display size on three parameters of reproductive success - the percentage of pollen removed, the number of pollen grains deposited on flowers entering the post-presentation phase, and the percent seed set. None of the relationships is significant (Table 1). However, in each case the sign of the coefficient is positive as expected from the prediction of Figure 3.

These measures show that the plant visitors are effective in carrying out pollination. By the time that a flower has entered the post-presentation phase, most of its pollen has been removed and there is much more pollen deposited on the stigma than is required to fertilise the number of ovules.

A flower would receive many visits during its lifetime if visits occur at the rate of one an hour as the presentation phase lasts as long as 14 hours of pollinator activity time (Chapter 3).

Thus pollinator service does not appear to be limiting. There is no particular relationship between the amount of pollen deposited on a flower and the amount removed (Figure 5).

The figures for the average percentage seed set show quite considerable variation between plants. This variation is apparently not due to pollinator limitation. Rather, it seems likely that plants differ in their ability to successfully mature seeds. This could either be due to variation in available resources for seed production, or to the effect of a powdery mildew that was evident on many plants in the 1988 flowering season. The result is consistent with the patchy results of hand-pollinations (Chapters 1, 2 and 5).

Table 1. The correlations between the size of the floral display and various measures relating to reproductive success.

Measure	n	Correlation with log (Total fls)
log (Bouts/hr, + 1), Day 1	30	0.4534*
log (Bouts/hr, + 1), Day 2	30	0.6148**
log (Bouts/hr, + 1), Day 3	32	0.7376**
log (Probes/bout)[1]	39	0.7526**
log (Probes/Presentation-Phase Fl.)[1]	39	-0.5400**
% Pollen Removed	38	0.0888
Pollen Deposited	38	0.1170
% Seed Set	31	0.0566

[1] Ignoring bouts where only one flower was visited.
* $P < 0.05$ ** $P < 0.01$

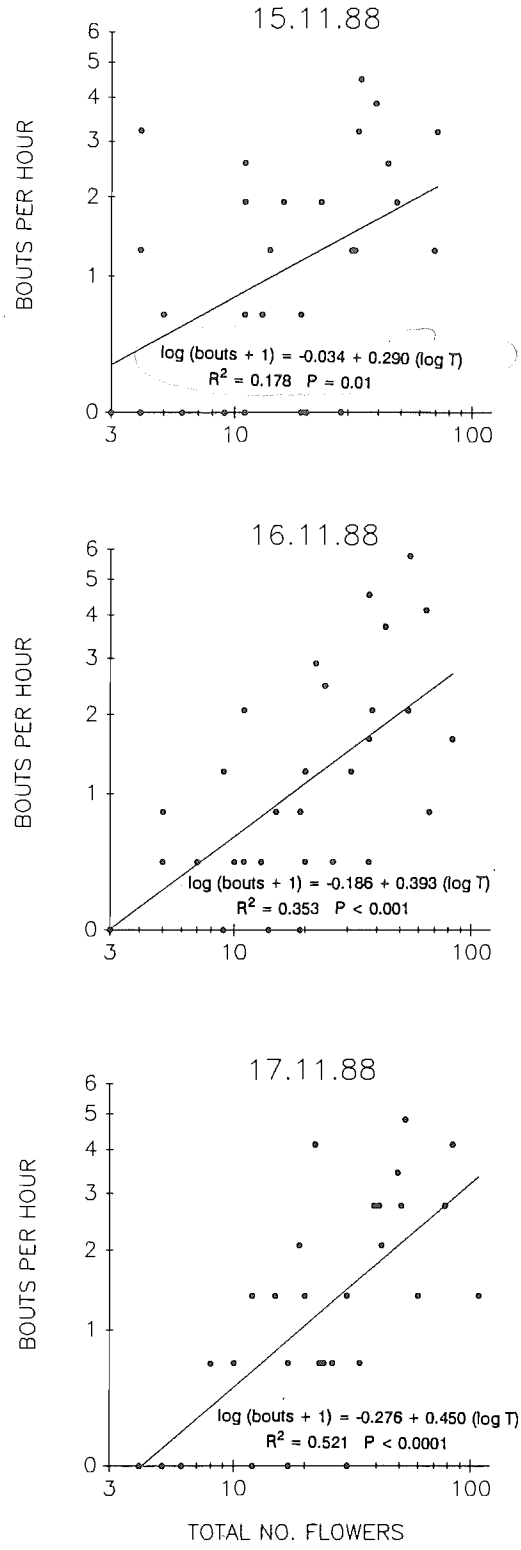


Figure 1 The relationship between the number of bouts (the number of times *Protohystricia* landed on a plant and began to visit flowers) and the total number of flowers presented (all phases) on three days for 32 plants. Single factor regression lines are shown. (T = Total flowers presented).

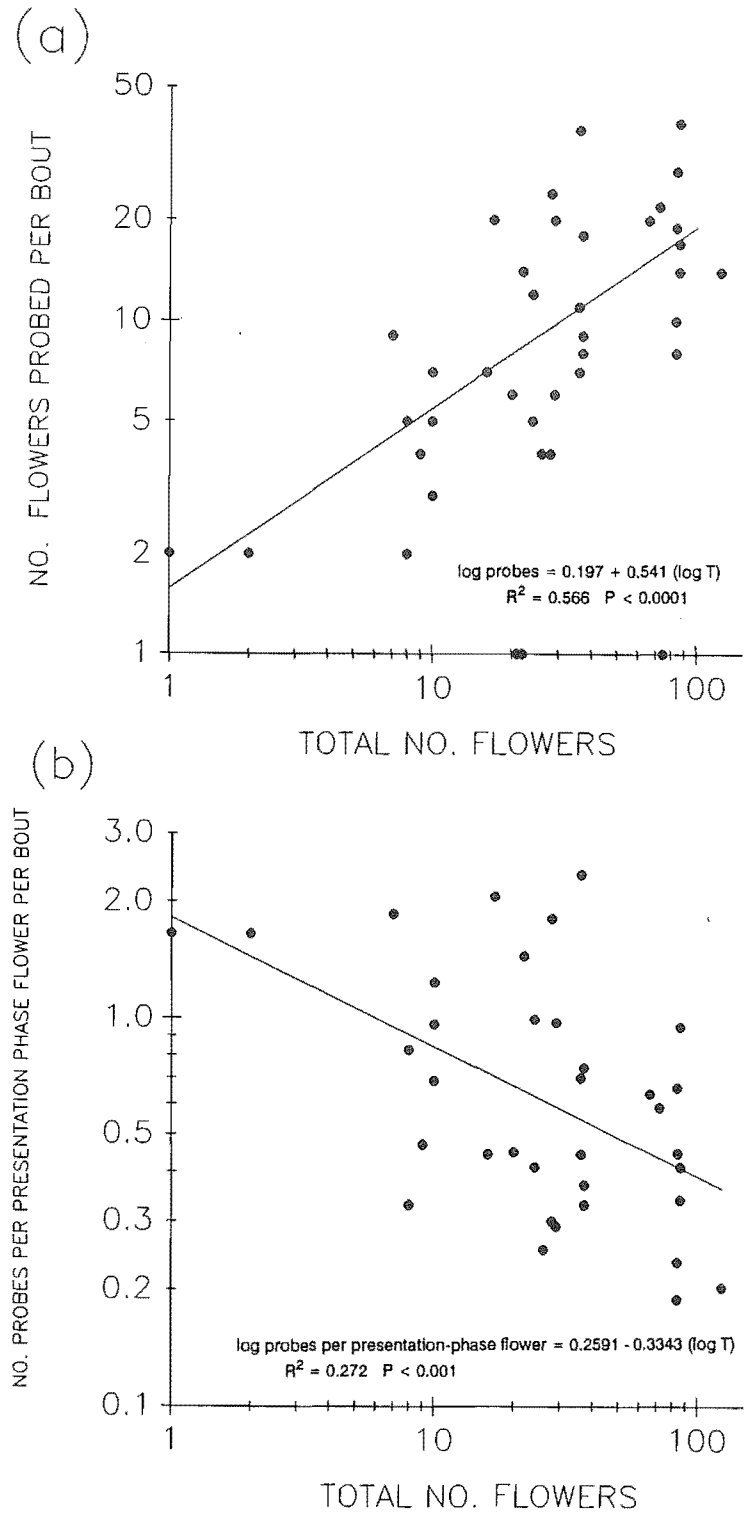


Figure 2. The relationship between the number of flowers probed in a single bout and the number of flowers presented (all phases) for 39 bouts. (The 3 bouts that resulted in visits to a single flower were ignored when fitting the regression lines).

a. The number of flowers probed and the total number of flowers.

b. The number of probes made per presentation-phase flower and the total number of flowers.

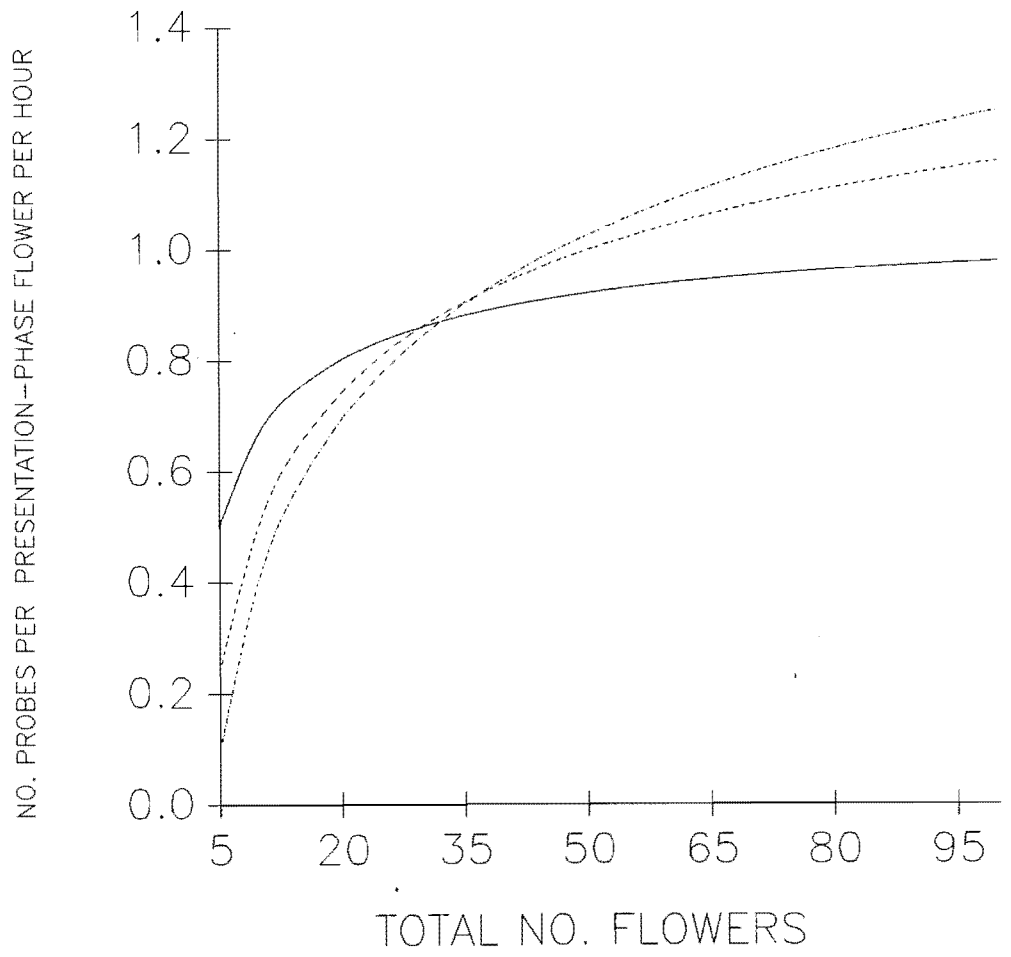


Figure 3 The relationship between the estimated number of probes to each receptive flower per hour and the total number of flowers presented (all phases) for the three days. The number of probes is estimated from the number of bouts per hour (Figure 1) and the chance of each receptive flower being probed in a single bout (Figure 2(b)). (15.11.88. - solid line; 16.11.88. - dashed line; 17.11.88 - dash-dot line).

Adaptive significance of the post-presentation phase flowers.

To determine the significance of the post-presentation flowers, an estimate of the potential effect of removing the post-presentation phase flowers on the rate of pollination to each flower can be made. For this purpose, we need to estimate the rates of visits to presentation-phase flowers when post-presentation flowers are present and compare this with the hypothetical rate when post-presentation flowers are absent, based on the effects of display size on visitation rates. A number of assumptions are made. Firstly, it is assumed that the number of bouts made to each plant depends primarily on the total number of flowers on a plant, not the number of presentation-phase flowers. This seems reasonable as the change from post-presentation to presentation phases is subtle (see Chapter 1) and it would be difficult for a pollinator to assess the number of presentation flowers until it is actually on the plant.

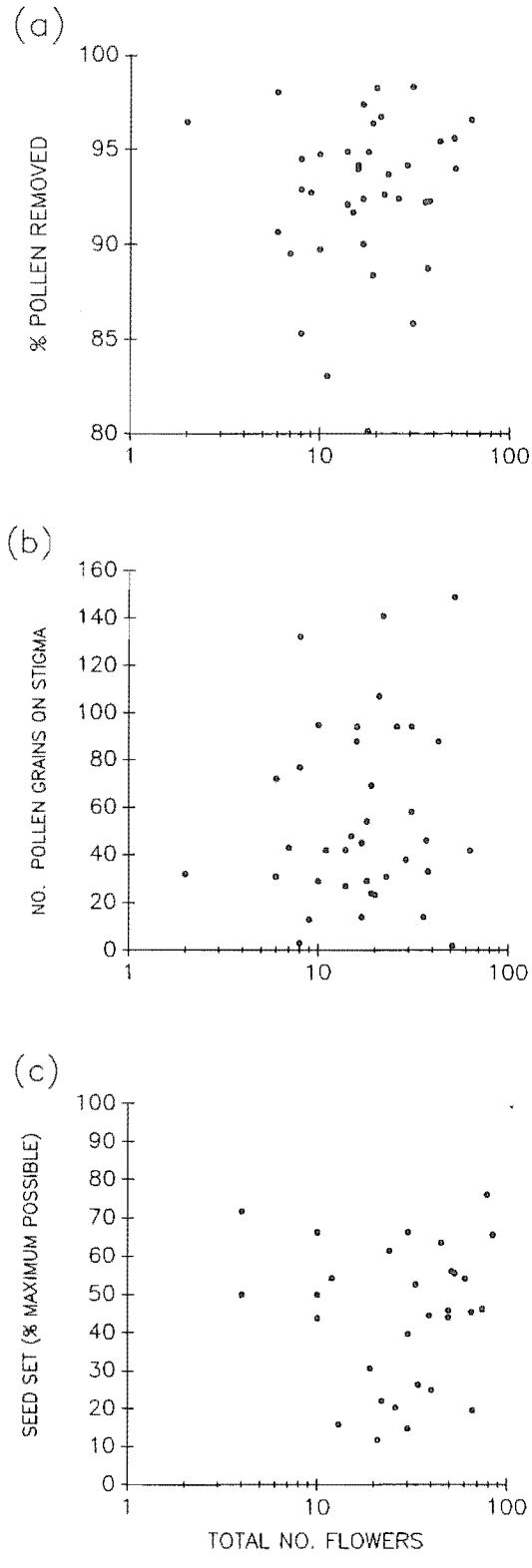


Figure 4 The relationship between three measures of reproductive success and the number of flowers presented (all phases).

a. The percentage of pollen grains removed from flowers by the time they enter the post-presentation phase.

b. The number of pollen grains on the stigma as the flower enters the post-presentation phase.

c. Seed set (percentage of the maximum possible).

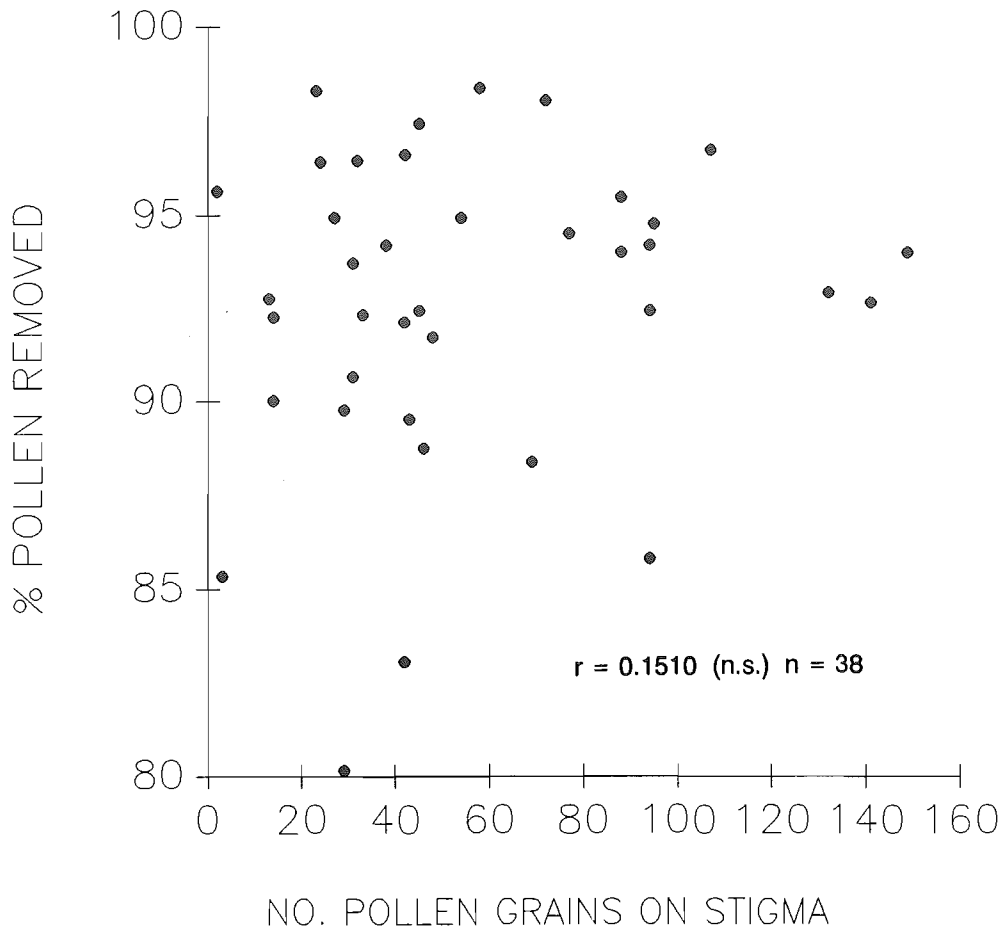


Figure 5. The relationship between male and female reproductive success (pollen exported and deposited) for flowers entering the post-presentation plants.

In addition, it is noticeable that as *P.huttoni* moves over a plant probing flowers, post-presentation flowers are often approached but not probed. Thus the number of flowers *encountered* exceeds the number probed. It is assumed here that the two types of flowers on a plant are encountered in proportion to their abundance. Moreover, it is assumed that the two flower types are arranged randomly so that it is not possible to predict the type of the next flower until it is encountered (see Chapter 6). Each presentation-phase flower encountered is assumed to be probed. If it is assumed that the proportion of the presentation-phase flowers is assumed to be 60.6% (see Chapter 2) and that 84.4% of the probes are made to these types of flower (Chapter 3), it is possible to estimate the flower encounter rate from the observed rate of probing. Finally, assuming that this rate holds for plants without post-presentation phase flowers, we can estimate the proportion of the remaining presentation phase flowers that is encountered and

probed. From this proportion and the rate of approach above, an hourly rate of visits per presentation-phase flower can be evaluated. The calculations may be shown as follows where

T = Total number of flowers on plant

E_T = the number of flowers encountered for a plant of size T

P_T = the number of probes made in a bout for a plant of size T

v = proportion of probes made to presentation-phase flowers (0.844)

j = proportion of presentation phase flowers (0.606)

R_{T+} = the number of probes per presentation-phase flower in intact plant of size T

R_{T-} = probes per presentation-phase flower in plant without post-presentation phase flowers of size T

B_T = number of bouts per hour to a plant of size T

H_{T+} = probes per presentation-phase flower per hour in intact plants of size T

H_{T-} = probes per presentation-phase flower per hour in plants without post-presentation phase flowers of size T

The encounter rate, the number of flowers encountered on a bout, is assumed to be directly proportional to the probe rate (correcting for probes made to post-presentation flowers). Hence,

$$E_T = \frac{P_T v}{j}$$

This encounter rate, is assumed to be the same for intact plants and those without post-presentation phase flowers. Holding the number of presentation-phase flowers constant and removing post-presentation flowers from one of a pair of plants would give Tj flowers on the manipulated plant. We will call this number S. The expected number of presentation-phase flowers encountered for an intact plant will be given by

$$R_{T+} = \frac{E_T j}{T}$$

while in manipulated plants by

$$R_{T-} = \frac{E_S}{S}$$

The hourly number of visits to each presentation-phase flower is simply

$$H_{T+} = R_{T+}B_T$$

and

$$H_{T-} = R_{T-}B_S$$

Figure 6 shows R_T - the number of probes per presentation-phase flower, and H_T - the hourly rate of probes per presentation-phase flowers for plants with a given number of presentation-phase flowers with or without post-presentation flowers. The rate of visitation per bout is estimated to be nearly the same for both situations (Figure 6(a)). However, because the display of the plants without post-presentation flowers is smaller, the approach rate per hour will be less. This has the result that the visitation rate to presentation-phase flowers per hour would be considerably lower per hour if there were no post-presentation phase flowers (Figure 6(b)).

Alternatively, the estimate of HT could be found for intact plants from the rate of RT found by directly measuring visits per presentation-phase flower as shown in Figure 3. Figure 7 shows the comparison of HT made this way with the estimate made in the previous figure. The two rates match very closely so give some confidence for the predictions made for plants without post-presentation phase flowers.

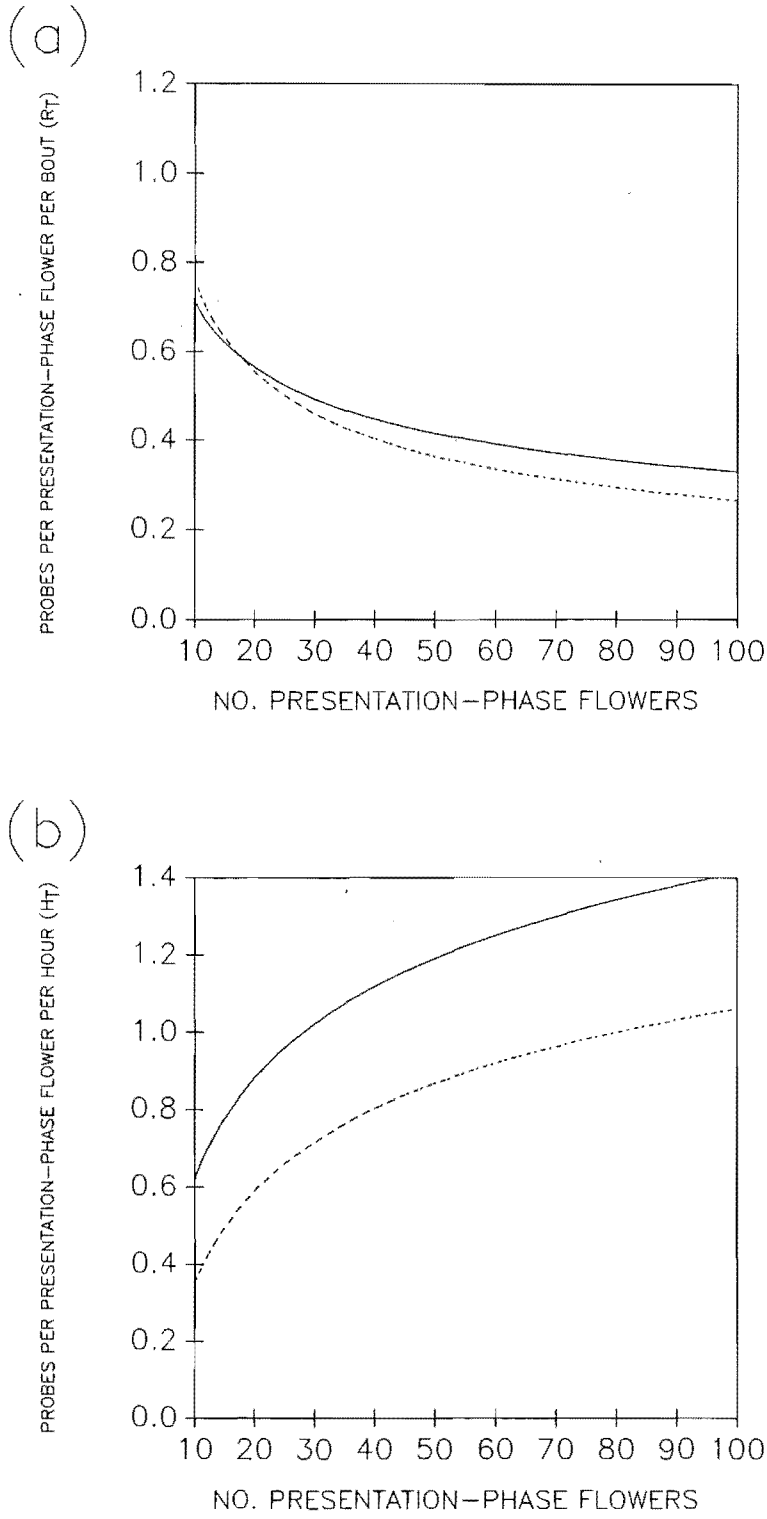


Figure 6. The relationship between the estimated visitation rates and the number of presentation-phase flowers for intact plants (solid lines) and the predicted relationship for plants with the post-presentation flowers removed (dashed line).

a. Visits per receptive flower during a single bout.

b. Visits per receptive flower per hour.

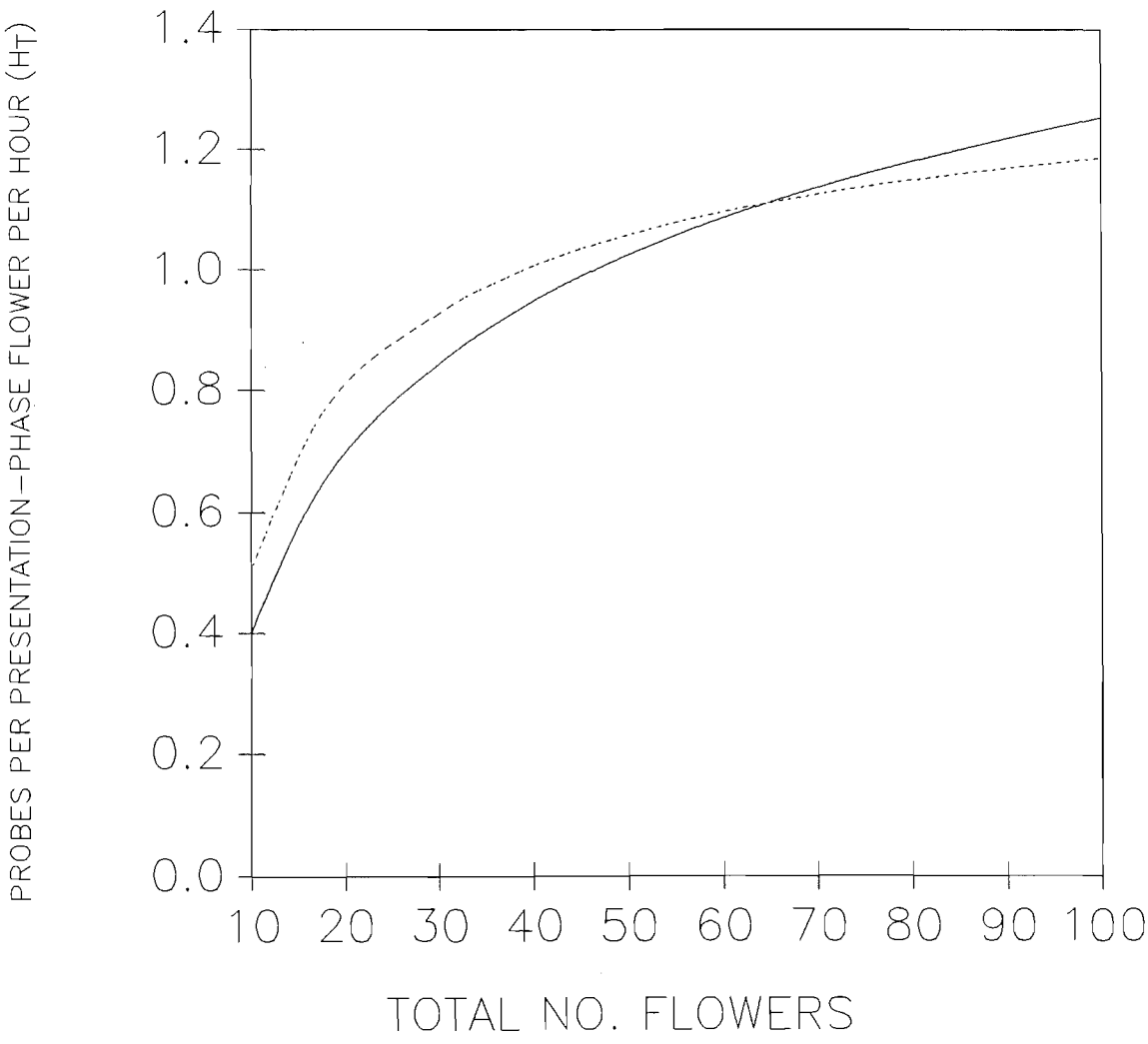


Figure 7. The estimated number of visits to each presentation-phase flower per hour based on the actual number of presentation-phase flowers (solid line) and the expected number of presentation-phase flowers (dashed line). This comparison is a check on the reliability of the prediction of visits to plants without post-presentation flowers made in Figure 6(b).

DISCUSSION

In accordance with many other studies that show that insects respond preferentially to larger targets (Bell 1985 and references therein), the data presented here show a significant size effect on the rate at which a plant is visited. Large plants experience more bouts per hour than smaller ones. However, the proportion of flowers visited at each bout declines as the size of the plant increases. The rate at which each individual flower is visited is the product of these two relationships. The combined effect demonstrates that the average rate at which an individual flower is visited per hour depends relatively little on the size of the plant that the flower is found

on. However, flowers on the smallest plants experience a lower number of visits per hour than larger plants; the effect is nevertheless slight.

The general independence of flower number and per-flower visitation rates is confirmed by the more direct measures of maternal and paternal reproductive success. Seed set per fruit, the amount of pollen deposited, and the amount removed from a flower over its lifetime, all show no significant effect of plant size. Virtually all flowers have large numbers of pollen grains on their stigmas by the end of the functional presentation phases. Similarly, all flowers had had at least 80% of their pollen removed by this stage.

It is hazardous to extrapolate the rates of pollen removal and deposition to the number of offspring left in the next generation. In particular, male reproductive success depends, on the number of stigmas reached by the pollen and the subsequent fertilisation of the ovules. It is possible that on occasion, flowers that have less pollen removed may actually have higher male success because more of their pollen is deposited successfully in the right place (Harder and Thomson 1989). Also, the rate of pollen removal may be more important than the absolute amount removed (see Chapter 3). A flower on a larger plant of *M.colensoi* may father more seeds because more vectors may move its pollen to receptive flowers.

Female reproductive success is more easily measured. The amount of seed set per fruit is a good indication of the number of potential offspring. However, the genetic quality of the seed is likely to be important. Self-pollen probably produces seeds of lower quality than outcross pollen (review in Charlesworth and Charlesworth 1987) and seeds from a single pollen donor are probably worse than those resulting from multiple-donor pollinations (Marshall and Ellstrand 1986). The genetic identity of the pollen on a stigma will depend to a large degree on the pollen carryover as well as pollinator behaviour and will be considered further in the next chapter.

Despite these limitations on the interpretation of the results, it is possible to examine their implications on plant and pollinator strategies. Firstly from the point of view of the pollinator, it is

of interest to examine the success of the foraging strategy. There is an expectation that pollinators should adopt a strategy of foraging that maximises their rate of energy gain. How do pollinators choose which flowers to visit? How much of the time should flowers on large plants be visited and how often should flowers on small plants be visited? The allocation of visitation time to plants of varying sizes may be thought of as an allocation strategy. Lloyd (1988) presents a general principle for allocation strategies. He predicts that at the ESS (the evolutionary stable strategy), the marginal gains from increasing each of the allocations (in this case, the amount of time spent at flowers of different plant sizes) are equal. One would expect that the time spent at each flower should be equal across all plant sizes, and that the number of visits each flower receives will also be equal. The "ideal-free distribution" postulated by Fretwell and Lucas (1970) is one case of Lloyd's more general marginal advantage theorem. They argued that the frequency at which different habitats are utilised by birds should depend on the amount of resources each source contains. An excellent example of equalised marginal gains from pollination biology is Heinrich's study of the nectar production rates of different species in a bog in Maine (Heinrich 1976). He showed that species differed widely in their rate of nectar production. This variation was paralleled by the rate of visitation by bees, which in turn led to the result that the average amount of standing crop was equalised across all species. In the present context, there is an expectation that flowers will be visited in proportion to the amount of reward offered. If pollinators in general visited flowers of larger plants more often, the expected standing crop of nectar in small flowers would be greater. This should result in a switch to the smaller plants. However, the situation is likely to be more complex than this. Pyke (1981b) predicted that larger plants should in general be favoured because the costly time spent moving between plants is minimised. Whether small plants are visited may depend on the relative costs of moving between plants. I would suggest that there will be a threshold of plant size below which inter-plant cost will exceed nectar gained. The density of plants will be an important parameter determining the size of the moving cost. In the present study, plant densities are high and the cost of moving presumably relatively low. The threshold at which plants are no longer worthwhile will be low. The fact even very small plants receive some visits (Figure 1) lends support to this idea. There is evidence, nevertheless, from the rates of visitation that flowers on small plants may

receive less visits than those on larger plants (Figure 3). As a result, flowers on these smaller plants may have a longer time to replenish their nectar and thus the standing crop of these flowers may be higher. This departure from equalised rewards per flower may be due to the extra cost of inter-plant movements. Alternatively, the lower rates of visitation may be due simply to the difficulty for the low-flying pollinators to locate these small plants with their higher rewards. Departures from optimised behaviour are beginning to be noted (Stephens and Krebs 1986) and may be due to the limited ability of pollinators to forage optimally.

Turning now to the plant's reproductive success in relation to floral display, it can be seen that the aggregation of flowers has little effect on reproductive efficiency. All measures of total reproductive success appear to increase with plant size. The total amount of pollen received and donated and seeds set all increase with flower number. However, the rate of increase in any of the parameters measured is not clearly more than a proportionate increase, so success per flower scarcely changes. There may, however, be a slight disproportionate gain in visitation rate which may be reflected in greater fitness gains to the male function because of diversity of export directions. It is thus possible that pollination success increases in ways that are not measured here. In addition, there is no evidence that extra non-fruiting flowers are produced to increase male success, as envisaged by Willson and others (Willson and Rathcke 1974, Willson and Price 1977, Willson 1979, Queller 1983, Piper and Waite 1988). Seed set as well as male success apparently both increase linearly with floral display. A plant would apparently maximise its fecundity by growing as large as resources will allow (cf Wyatt 1980), but if it can only manage a small display, the success of each of its flowers will be similar to a larger plant. These results parallel other recent results on the pollination of species with free pollen (Andersson 1988, Schmid-Hempel and Speiser 1988, Klinkhamer et al. In press).

Although no experimental test of the adaptive significance of the post-presentation flowers was carried out, their presence on the plant may increase the rate of visitation to the presentation-phase flowers (Figure 6). Certain assumptions were made, however. In particular, it was assumed that pollinators do not alter their behaviour on plants without post-presentation

flowers. These plants would have greater rewards per flower on average and may therefore have longer bouts. The appropriate experiments that involve removal of post-presentation flowers and the subsequent monitoring of the *rates* of pollen removal and deposition measured are clearly needed to confirm this expected result. Such confirmation has been provided in two recent cases (Cruzan et al. 1988, Gori 1989) but not in others (Casper and La Pine 1984, Delph and Lively 1985, 1989). The importance of directing pollinators away from unrewarding flowers is undoubtedly important in understanding the function of post-presentation flowers. Why spent flowers are retained rather than simply abscised, however, may have several answers. They may, as suggested here, promote greater visitation rates per hour to the functional flowers (Cruzan et al. 1988, Gori 1989). Alternatively they may have no particular function and be present as inherited features from ancestors. In the present context it may be of interest to note that among species of *Myosotis,* even species that are apparently always autogamous also signal spent flowers by a colour change (see Chapters 1 and 2). Delph and Lively (1985, 1989) argue that in *Fuchshia excorticata,* old flowers are unable to be abscised straight away, as to do so would result in the loss of the style before the pollen tubes can reach the ovary.

In summary, the floral display is best viewed as an inevitable result of selection for maximum offspring quantity. The aggregation of flowers may have no particular consequences on the success of either male or female functions on a per flower basis. Small plants may have a lower visitation rate per flower per hour though this apparently does not cause any limitation on pollen export or receipt. Geitonogamy, however, increases with plant size (next chapter) and may be a factor not considered here that limits female success in large plants (Wyatt 1981). The retention of spent flowers may increase the rate at which pollen transport occurs. This may increase male success, through increased pollen-transporting events, and female success through increased mate choice.

CHAPTER FIVE

THE RELATIONSHIPS BETWEEN FLORAL DISPLAY SIZE, POLLEN CARRYOVER AND GEITONOGAMY IN *M.COLENSOI*

One important consequence of having a large number of flowers on a plant at any one time is that a large proportion of visits made by pollinators are likely to occur between flowers on the *same* plant, resulting in geitonogamous self-pollinations rather than cross pollinations (Beattie et al. 1973, Primack and Silander 1975, Frankie et al. 1976, Primack 1979, Schemske 1980b, Schmitt 1980, Hessing 1988). In a self-incompatible plant, this may result in clogging of the stigmas with self-pollen (Bawa and Opler 1975, Yeo 1975, Shore and Barrett 1984) and in pollen wastage (Baker 1964, Rathcke 1983), while in self-compatible species it may also reduce outcrossing and hence offspring quality and variability (Darwin 1876, Charlesworth and Charlesworth 1987, Hessing 1988). On the other hand, producing more flowers would obviously allow more offspring to be produced and may lead to more pollinator visits per plant and per flower (see Chapter 4). Geitonogamy presumably increases when more flowers are available. The effect of increased geitonogamy with increased floral displays may therefore be considered an unavoidable detrimental effect of large displays. This conflict between offspring quantity and quality has been postulated to impose an upper limit on the size of floral displays (Wyatt 1980, Hessing 1988). Lloyd (1987 has shown that geitonogamy can never be selected for as it retains the cost of advertising without gaining the benefit of increased cross-pollination.

It will be argue here that pollen carryover may reduce the postulated disadvantage of a large floral display. Geitonogamy, thus, can, to some extent, be controlled by plants.

Until recently it was often assumed that a pollinator deposited all the pollen it had picked up at one flower on the stigma of the next (eg Levin and Kerster 1968, Beattie et al. 1973, Beattie and Culver 1979). This would mean that only the first flower of each plant visited would receive

outcrossed pollen. However recent work has shown that pollinators frequently deposit only a fraction of the pollen they are carrying on the next flower (see Table 1 below). This is known as pollen carryover. This results in pollen loads of mixed paternity (Marshall and Ellstrand 1985) and allows pollen to be transported from one plant to several subsequent flowers on new plants. Other work has shown that populations are genetically more heterogeneous than we would predict from the data we have on pollinator movements and that this difference is in part the result of pollen carryover (Schaal 1980, Ennos and Clegg 1982, Handel 1982).

The frequency of geitonogamy has been documented only occasionally, and even fewer studies relate the frequency to plant size. Waller and Knight (1989) estimated the average rate of geitonogamous self-fertilisation in populations of *Impatiens*. They found that geitonogamy accounted for between 30% and 70% of the progeny. They speculated that the observed variation between populations may be due to differences in average plant size or to the behaviour of each population's pollinators. Peakall (1989) stained pollinaria of an orchid and tracked the natural dispersal. He estimated that about 22% of pollinations were geitonogamous. Crawford (1984) used a genetic marker to estimate outcrossing in *Malva moschata.* He demonstrated a positive relationship between flower number and estimated selfing rate and attributed this to increasing geitonogamy. Hessing (1988), using dye analogs of pollen, found that little dye was transported to other plants and that as plant size increased there was a weak increase in the frequency of geitonogamy. However, Geber (1985) has shown that the frequency of self pollination in *Mertensia* is almost independant of flower number. She demonstrated instead that larger plants receive only fractionally more intra-plant visits than small ones, that there is relatively high pollen carryover and that as a result there is no effect of plant size on levels of geitonogamous self-pollination.

The likely level of geitonogamy will depend not only on the numbers of flowers visited within a plant at each bout, but also on the amount of pollen carryover in the system. In this chapter, I develop a probabilistic model to evaluate the relative contributions of within-plant pollinator movements and carryover on the rate of self-pollination and use it, as well as data on

carryover and within-plant visits in *Myosotis colensoi*, to show that pollen carryover is a major factor affecting insect-mediated self-pollination. I will also show that carryover is partly under the control of the plant and that this ability to control carryover and in turn geitonogamy (and other aspects of pollen travel) is likely to be a major evolutionary force shaping the floral characteristics of plants.

METHODS

Pollen Carryover

On 29 November 1988, a group of plants were covered with a fine mesh material to keep pollinators away from the plants. All flowers that had already opened were removed from each plant. On successive days, the anthers were carefully removed as the flowers opened. As a result, each flower on a treated plant presented only a fresh unpollinated stigma. Each flower was marked by a unique combination of dyed toothpicks. On the afternoons of 2-4 November 1988, the mesh cages were removed and the flowers exposed for visits. Individuals of *P.huttoni* were allowed to forage in the area on unmanipulated plants of *M.colensoi*. As an individual fly began to forage on a treated plant, each flower that was probed and the order in which the flowers were probed was recorded. After the fly had left, each flower that had been probed was collected and placed into a small glass vial containing FAA.

Later, the amount of pollen deposited on the entire flower was estimated in the laboratory,using the whole-flower method used for obtaining pollen-ovule ratios, as described in Chapter 2. The amount of pollen on each flower was converted to a standard proportion by dividing by the maximum amount of pollen deposited at any flower in that run. The standardisation allows comparisons to be made between runs even when pollinators are carrying different sizes of pollen load (Waser and Price 1984).

THE MODEL AND BASIC ASSUMPTIONS

I present below a probabilistic model that simulates a pollinator visiting a number of flowers on a plant before moving on to visit other plants. Some of the pollen it deposits will be from other plants and some will be from within the plant being visited. I have assumed, partly for the sake of simplicity and partly because it gives the best fit to *most* available data (see discussion below), a model of **exponential decay**. It is assumed that:- (1) A pollinator approaches a plant fully loaded with pollen (the "pollen pool"); (2) Each flower on a plant is hermaphrodite; (3) At each flower, a visitor picks up and deposits pollen in equal amounts, ie pollen pickup and redeposition is in equilibrium; (4) This amount is a constant fraction of the pollen in the "pool" (the amount of pollen held on a pollinators body); (5) Pollen is deposited on the stigma of a flower before contact is made with the anthers; (6) Pollen is mixed thoroughly into the pool and (7) There is an equal likelihood of any pollen grain being deposited on the next flower.

Under this model, outcross pollen is deposited at a negatively exponential(declining) rate, after each successive within-plant flower is visited. Pollen carryover (**C**) is defined as the constant proportion of pollen retained on the pollinator at each visit. The relationship, between the proportion of "outcross" pollen (that from other plants) and the position of a flower in a sequence of geitonogamous visits is simply:-

$$O = C^{n-1} \qquad (1)$$

(where **O** is the proportion of outcross pollen deposited, **C** is the estimate of carryover, and **n** the position of the flower in the sequence).

Such a relationship is shown in the curves in Figure 1(a) for five different levels of carryover. It can be seen that the amount of outcross pollen deposited quickly reduces to a very low level after several flowers have been visited. For carryover figures above 0.80, however, there is a dramatic increase in the amount of outcross pollen, even after 20 visits.

Deriving an average measure for each bout

If a pollinator visited say 5 flowers on a plant before moving on, it would deposit only outcross pollen on the first flower (ignoring the pollen deposited from the flowers own anthers to its stigma). On the second flower it would leave outcross pollen at the proportion of foreign pollen left on its body - C, the third at C^2, the fourth at C^3 and the fifth at C^4. On *average* then, he has deposited outcross pollen equal to the sum of the individual terms divided by the number of flowers visited, or:-

$$O=(C^0+C^1+C^2 \ldots C^N)/N \qquad (2)$$

(where N = the number of flowers visited in a bout).

The numerator in this expression forms a geometric series and can be rewritten as:-

$$(1-C^N)/(1-C) \qquad (3)$$

and represents the cumulative amount of foreign pollen deposited.

The average amount of outcross pollen deposited therefore becomes:-

$$O = \frac{(1-C^N)/(1-C)}{N} \qquad (4)$$

Figure 1(b) shows this relationship under the same levels of carryover. The curves reveal that the average proportion of outcross pollen after *x* visits is higher than the proportion deposited on the *x*th flower, as expected. All degrees of carryover increase outcrossing but the affect is greatest at the highest value.

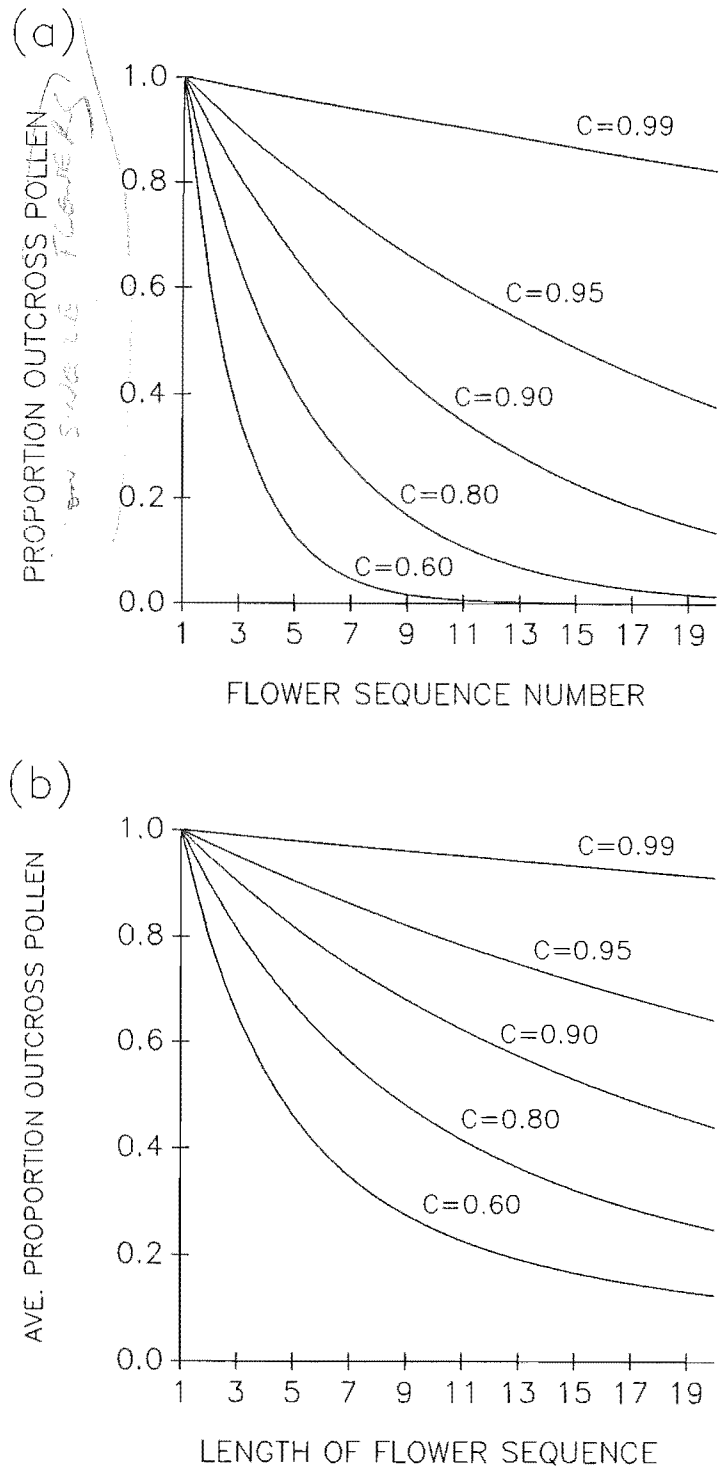


Figure 1. Pollen carryover and within-plant bouts.

a. The relationship between the position of a flower in a sequence of within-plant visits and the proportion of xenogamous pollen deposited on it. Different levels of pollen carryover (*C*) are shown.

b. The relationship between the average amount of xenogamous pollen deposited during a bout and the length of the bout (the number of flowers probed) under different levels of pollen carryover.

Justification of the Model.

Several other workers have suggested models of pollen carryover (Bateman 1947, Levin and Kerster 1971, Silander and Primack 1978, Waser 1978, Crawford 1984, Geber 1985). Most use models of exponential decay. Recently, however, objections have been made about the use of an exponential model for carryover (Price and Waser 1982, Lertzman and Gass 1983). Lertzman and Gass (1983) discussed alternative models of pollen carryover and suggested that the exponential decay model is over-simplistic and doesn't fit the available data well. They assert that a fixed stigma capacity for pollen, layering of pollen from successive donors and occasional failures to contact the stigma or anthers should act to give irregular and linear pollen carryover. Waser and Price (Price and Waser 1982, Waser and Price 1982, 1984) have suggested that a linear model fits their data on hummingbird pollination of *Delphinium* and *Ipomopsis* better than an exponential one.

While it is accepted that an exponential model may be simplistic for stochastic reasons, the influence of stochastic events will tend to increase the variance around what is essentially a variable but still approximately exponential curve. Moreover, the bulk of the available data does not support the linear model. Several recent studies with a number of different plants and pollen vectors have measured pollen carryover . The results of these studies are presented in Table 1. Several different methods of measuring carryover have been used in these studies including heterospecific pollen donors, dye analogues, emasculated flowers, a natural pollen polymorphism, and calculation of the ratio of pollen in the "pool" and the amount deposited on stigmas (this last estimate is likely to overestimate carryover as it assumes that all pollen in the pool is available to be deposited and that pollen is deposited only on the stigma and not elsewhere on the flower or lost). One study (Waddington 1981) even used a dead pollinator to artificially probe flowers. Only 9 of the studies cited contained enough information to test the fit of exponential against linear models. A linear model explained more of the variance than an exponential model in only two studies. For one of these studies, (Price and Waser 1982), the difference in R^2 is only very slight. In the other, bees visiting *Stellaria pubera* (Campbell 1985), the difference is still small.

Table 1. Pollen carryover in different species and pollinated by various vectors.

SPECIES	VECTOR	METHOD*	MODEL	R^2	CARRYOVER(C)	SOURCE
Trifolium pratense	Bee	a	-	-	99.5	Plowright and Hartling (1981)
Ipomopsis aggregata	Hummingbird	b	lin exp	0.70 0.67	94.7	Price & Waser (1982)
Linaria vulgaris	Bumble bee	c	exp**	-	92.0	Thomson (1986)
Mertensia ciliata	Bumble bee	c	exp**	-	90.5	Geber (1985)
Myosotis colensoi	Tachinid fly	c	lin exp	0.64 0.66	89.8	This study
Epilobium angustifolium	Bumble bee	c	lin exp	0.15 0.36	85.6	Galen & Plowright (1985)
Stellaria pubera	Beefly	c	exp		84.4	Campbell (1985)
Scleranthus perennis	Ant	b	lin exp	0.79 0.85	81.7	Svensson (1985)
Clintonia borealis	Bumble bee	c	lin exp	0.75 0.88	81.5	Thomson & Plowright (1980)
Oenothera fruticosa	Bee	a	-	-	79.7	Silander and Primack (1978)
Oenothera fruticosa	Bee	a	-	-	71.2	Primack and Silander (1975)
Claytonia virginica	Beefly	c	exp	-	71.2	Campbell (1985)
Diervilla lonicera	Bumble bee	c	lin exp	0.63 0.80	68.7	Thomson & Plowright (1980)
Erythronium americanum	Bumble bee	d	exp**	-	64.0	Thomson (1986)
Oenothera fruticosa	Beetle	a	-	-	63.5	Primack and Silander (1975)
Delphinium virescens	Bumble bee	f	-	-	61.9	Waddington (1981)
Stellaria pubera	Bee	c	lin exp	0.67 0.51	53.3	Campbell (1985)
Phlox glaberrima	Lepidoptera	e	lin exp	0.90 0.97	50.2	Levin & Berube (1972)

* a - ratio of pollen load to deposition
b - dye analog
c - emasculated flowers
d - pollen polymorphism
e - heterospecific pollen
f - dead pollinator

** Author indicates an exponential (exp) model gave a better fit than a linear one (lin).

For all other studies for which I have data, or the authors themselves have done a similar analysis (see Table 1), the exponential model provides a better fit than a linear one. It appears that the exponential model provides a useful approximation of pollen carryover for the majority of systems studied so far.

RESULTS AND DISCUSSION

Myosotis colensoi

Figure 2 presents the results of the pollen carryover experiment. Figure 2(a) shows the raw data and Figure 2(b), shows the mean for each position in a sequence. The amount of pollen deposited on a flower is very variable but declines as more flowers are visited. This decline in the average proportion of pollen deposited is fitted slightly better by an exponential rather than a linear rate of decay, although for both models the fit is highly significant (linear $R^2 = 0.643$ $p < 0.001$; exponential $R^2 = 0.656$ $p < 0.001$; $n = 13$).

The relationship between the natural logarithm of the average proportion of pollen deposited and the position of the flower in the sequence is given by the following equation:

$$\text{Ln (standardised average pollen deposited)} = -0.108n - 0.458$$

The best estimate for C the carryover proportion is simply $e^{-0.108}$ which is 0.898. This means that on average, 90% of the pollen on a pollinator's body is retained during a visit to a flower. The number of flowers probed for plants of a given size T was found in the previous chapter.

$$\log (N) = 0.197 + 0.541\log(T)$$

Applying equation (4) above, the average amount of outcross-pollen deposited on a plant of size T can be estimated. The estimated proportion of outcross pollen on a plant of a given size is shown in Figure 3. Geitonogamy is expected to increase with an increase in plant size but because of a high level of pollen carryover and the decreasing proportion of flowers on a plant visited in a single bout, the decline is relatively slow .

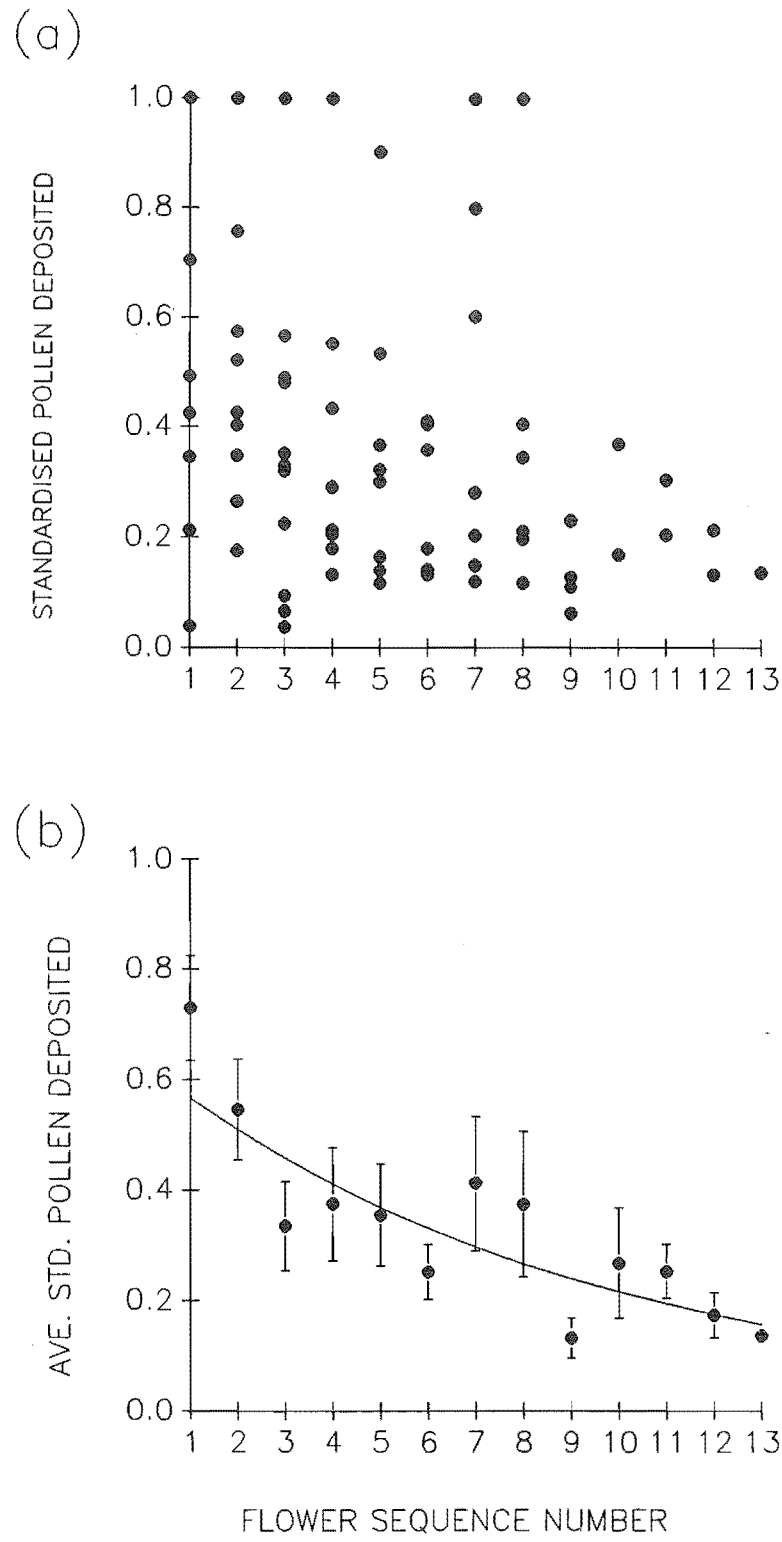


Figure 2. Pollen carryover in *M.colensoi* by the fly *P.huttoni*.

a. The relationship between the total amount of pollen deposited anywhere on the flower and the position of the flower in the sequence.

b. The averages from Figure 2(a) and the exponential curve found by regression of the natural logarithm of pollen deposited against the position of the flower in the sequence.

The net result of the combined estimates of pollen carryover and geitonogamous plants reveals that geitonogamy is important in *M.colensoi*. It increases with plant size and may account for as much as 50% of all pollinations in the largest plants. Considering the large size of floral display of many of the plants in a population and the habit of pollinators visiting many flowers on a plant, however, this observed estimate of geitonogamy may seem surprisingly low. The relatively high level of carryover (close to 90%) that apparently occurs when *P.huttoni* visits a plant is clearly important. Figure 3 also presents parallel estimates of outcrossing when the level of pollen carryover is varied. Factors that influence carryover will obviously be very important in controlling geitonogamy and may be under strong selective pressures. It will be shown below that some plant characteristics may be important in determining pollen carryover and hence the likelihood of widespread geitonogamy.

Other plant-pollinator systems.

Table 1 gives an estimate for C, the percentage carryover described in the model above, for as many other plant-pollinator systems as I could find data for in the literature. This parameter was estimated in each case using the method described above for *M.colensoi*. In some cases, this line was fitted by the authors of the original paper (Price and Waser 1982, Campbell 1985, Galen and Plowright 1985), while in the others, where raw data was available, I did the analysis myself. In each case deposition was standardised in the way described above except for the study by Campbell (1985 who standardised on the first flower in each case and the study by Galen and Plowright (1985) who don't appear to have used any standardising procedure).

Pollen carryover (C) ranges from 94.7 % to 50.2%, almost doubling between the smallest and largest values. This range of carryover is probably conservative and values even outside these figures could be expected for some systems.

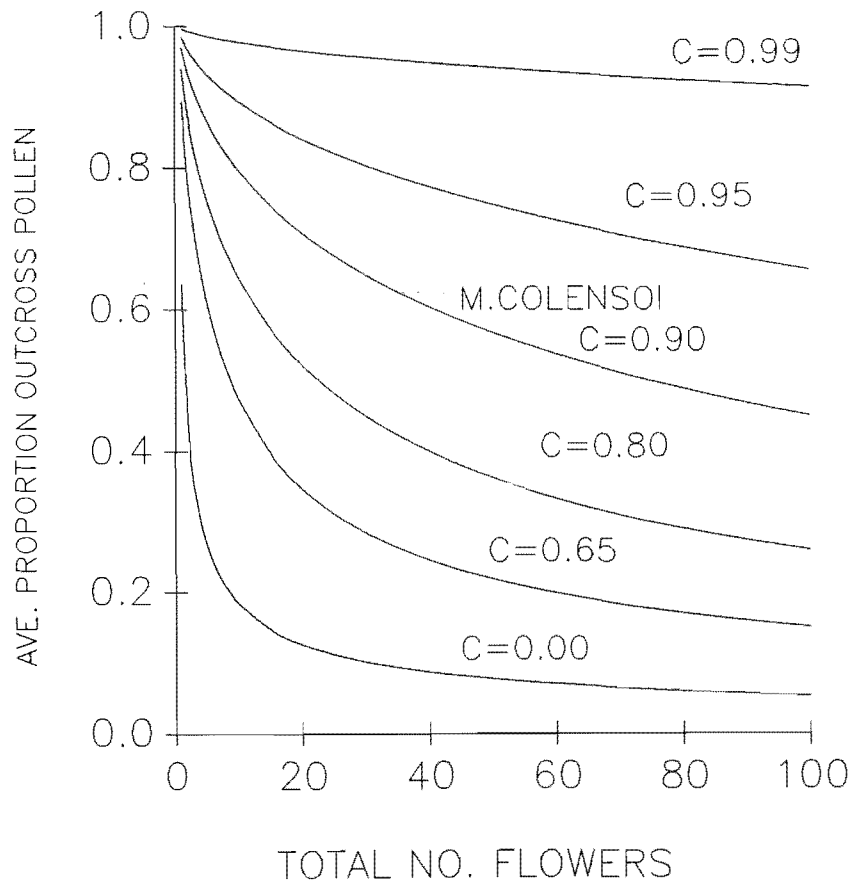


Figure 3. The expected affect of altering the level of pollen carryover on the average amount of xenogamous pollen received during an average-length bout. The expected relationship for *M.colensoi* is also shown.

Flower Number and Within-plant Visits

It is often argued that with an increasing number of flowers on a plant, an increase in within plant visits also occurs (e.g. Arroyo 1976, Frankie et al. 1976, Augspurger 1980). If a pollinator visits all the flowers open on a plant, then it follows that xenogamy (the deposition of outcross pollen) can be estimated directly from Figure 1(b). Generally, however, pollinators visit only a fraction of the flowers available on a plant and therefore in order to estimate levels of xenogamy on a plant of a given size, one must be able to predict the number of visits that will occur within that plant. This has been documented in several recent papers (Zimmerman 1981, Bell 1985, Geber 1985, Andersson 1988, Schmid-Hempel and Speiser 1988, Klinkhamer et al. In press and see Chapter 4). All these studies show that rather than increasing in proportion to the number of available flowers, the number of flowers probed at a single bout increases at a

decelerating rate. In other words, the chance of an individual flower being visited during a single bout decreases with increasing plant size.

Other studies have measured how many visits an average pollinator makes to an average sized plant, and confirm that pollinators usually visit a relatively small number of flowers at a single bout (eg. Augspurger 1980, Schemske 1980b, Paton 1982, Delph and Lively 1985, Ramsey 1988). For example, Augspurger (1980) found that despite individuals of *Hybanthus prunifolius* bearing on average over 200 flowers at once, pollinators visit on average less than 10 of these at a single bout. Similarly, Schemske (1980b) showed that *Combretum farinosum* has up to 4000 flowers open at once, but that hummingbirds visit an average of only 11.8 flowers before leaving a tree.

It appears, therefore that with the exception of isolated plants (Frankie et al. 1976, Zimmerman 1981), that pollinators make relatively short visits within a plant and that even on mass-flowering plants, the number of flowers visited is characteristically in the order of tens rather than hundreds before moving on to another plant. This result has lead to speculation, particularly for tropical mass-flowering species, about the reasons pollinators move to new patches rather than visiting more flowers on a plant (see Chapter 6). Some of the reasons that have been given (reviewed in Stephenson 1982) include nectar satiation, mass depletion of nectar by flocks of pollinators, and predation or aggressive behaviour by other animals.

Whatever the reason, the result of these regular movements between plants, coupled with a moderate level of pollen carryover may lead to a large proportion of xenogamous pollinations. Thus in the case of Augsperger's *Hybanthus* pollen carryover of 85% (probably an underestimate for a hummingbird pollinated plant) would allow 23.2% of the pollen deposited on even the tenth flower to be outcross pollen and on average 53.5% over ten flowers. To ignore the importance of pollen carryover (or to assume as she did that carryover was probably low - around 10%) would lead to seriously underestimating the likely level of cross-pollination.

The above discussion of pollen carryover and the frequency of within-plant visits from other plants shows that the conclusions reached for *M.colensoi* may be fairly general. In particular, pollen carryover may often be high. The dilemma of increasing geitonogamy with increasing plant size may be partly relieved.

The control of Geitonogamy - pollinator behaviour and plant characteristics.

Having established a basic model and identified the two basic parameters - within-plant visits and pollen carryover, we can begin to examine what characteristics of pollinator behaviour and floral organisation influence these parameters.

(1) Flower specialisation and pollinator constancy.

Factors that promote the variability of placement of pollen on the pollinator body will directly influence carryover (Price and Waser 1982, Lertzman and Gass 1983, Waser and Price 1984). Placing pollen over a larger more variable area will decrease the proportion of that area that contacts the stigma or other flower parts at each visit. This will mean that a smaller proportion of pollen will be deposited on each flower and carryover will therefore increase.

If a pollinator approaches a flower in a variable manner, pollen may be loaded onto the pollinator in a variable way over much of the pollinator's body. Pollen carryover will therefore be enhanced relative to that resulting from a pollinator that approaches the flower in a consistent manner. Flower features that promote approach from all directions will lead to higher carryover. Unspecialised blossoms like those of the Ranunculaceae are examples of such a flower type. Ants (Svensson 1985, 1988) may cause relatively high level of pollen carryover (Table 1) because the dish-shaped blossom is crawled over in a variable manner, and the body of the ant contacts stigmas and anthers in a erratic way. However, many unspecialised flowers are likely to be visited by equally unspecialised pollinators. These type of pollinators will also tend to be inconstant and frequently visit flowers of another species which will cause many interspecific pollinations if carryover is high. Other mechanisms that promote variable pollen loading without utilising unspecialised pollinators are likely to be more successful at promoting intraspecific

outcrossing than a variable approach system.

(2) Pollinator Size

A large pollinator will be able to carry a larger pollen load than a small one. For example, birds that visit flowers and carry pollen on a generalised area such as the head, will carry large loads of pollen. The ratio of pollen deposited at a single visit to the amount in the pollen pool is likely to be small and the pollen carryover larger than for a flower utilising smaller pollinators e.g. Table 1 - *Ipomopsis* visited by hummingbirds (Waser and Price 1984).

(3) Grooming and pollen layering

Many pollinators, especially bees and flies regularly groom pollen from their bodies (Thomson,JD and Plowright 1980, Thomson,JD 1986). This will have a profound effect on pollen carryover as the pollen pool will be constantly being reduced and therefore pollen carryover shortened. Thomson (1986) showed that in runs where bees groomed between flowers there was less carryover of *Erythronium* pollen than in runs where no grooming took place. On the other hand, grooming may well uncover previously buried pollen and expose it to stigmas, thus enhancing pollen carryover if pollen is deposited in layers that are usually successively covered over (Lertzman and Gass 1983). On occasions, some pollen may travel for a long way on a pollinator before being deposited after grooming has mixed the pool. The extent of pollen layering and pollinator grooming and their general importance in pollen carryover remain largely unknown.

(4) Handling time and reward levels

The average time spent at a flower will determine, to a large extent, the amount of turnover of pollen that occurs at each flower. A relatively longer handling time will presumably lead to more turnover and less carryover. The amount of reward offered at a flower has been shown to be a major determinant of handling time and the amount of pollen deposited. (Thomson,JD and Plowright 1980, Waddington 1981, Galen and Plowright 1985, Thomson,JD 1986 but see also Inouye 1980). The amount of reward offered may also alter the number of

flowers visited within a plant (Waddington 1981, Galen and Plowright 1985) as is expected under Charnov's marginal value theorem (Charnov 1976). Plants may thus be able to influence both the amount of pollen carryover and the amount of within-plant visits by altering the amount of rewards offered. In particular, if the outcrossing is important, as little reward as possible should be offered at once. As long as pollinator visitation rates are not limiting, less reward may lead to higher carryover and smaller amounts of pollen deposited and picked up at each visit. There is some evidence that small loads of pollen are deposited more efficiently than large loads (Harder and Thomson 1989) and so male fitness as well as female fitness may benefit from increased carryover

(5) Anther variability

If a plant is able to vary the spatial position of its anthers, it may be able to increase the area of the pollen pool on the pollinator. This variation could be between or within flowers or plants. Where pollinators have a large potential pollen collecting surface, such as birds or mammals, anthers could be varied in position so as to maximise deposition area. By a similar argument to that extended for flower specialisation and flower constancy above we can see that increasing this deposition area may be expected to produce more carryover. Waser and Price (1984) manipulated inflorescences of *Ipomopsis* by changing the amount of spatial variability of flowers by removing anthers from selected positions. They found that pollen carryover was indeed maximised when the spatial variability of anther position was enhanced. They also measured the amount of variability found naturally in the population and found significant within- and between-plant variation. Such variation is likely to be of major significance if within-plant visits are important in selection. Many bird-pollinated plants demonstrate considerable variation in the heights of the anthers relative to the floral tube (pers obs, see for example Grant and Grant 1968). I would also suggest that this variation exists, at least in part, as a mechanism to spread pollen deposition and enhance pollen carryover.

CONCLUSIONS

The expected result that geitonogamy increases with plant size has been confirmed for *Myosotis colensoi*. Large plants are expected to receive about half of their pollinations from other flowers on the same plant. However, I have attempted to show in this paper that there are two important parameters that control the rate of geitonogamous self-pollination in hermaphrodite plants. They are the rate at which pollinators visit flowers on the same plant and the rate of pollen carryover. I can find no hard evidence to show that pollinators do spend very long periods of time on plants with large displays, though this is frequently assumed. Moreover, the rate of pollen carryover has been frequently underestimated. The result of these assumptions is that there has been a tendency to suppose that mass flowering plants are likely to suffer high levels of inbreeding depression if self-compatible, or be rather inefficient in ensuring successful pollinations if incompatible.

Some mechanisms have been identified that allow a plant to escape high levels of geitonogamy including manipulating reward levels to pollinators and varying the position of anthers. A critical look at the evidence available indicates that within-plant visits usually do not increase proportionately with the size of the floral display. Moreover, pollen carryover in most situations is large enough to allow a percentage of outcross pollen to be deposited on all flowers visited. Results from *M.colensoi* and *Mertensia* (Geber 1985) support these ideas. If any degree of differential pollen tube growth or mechanisms of male-male competition in the stigma and style (for a review see Stephenson and Bertin 1983) it is possible that all offspring will be outcrossed, even in plants that do not show the features usually considered to promote it. Such cryptic self-incompatability has been demonstrated several times in different taxa (Bateman 1956, Weller and Ornduff 1977, Bowman 1987, Beccera *pers. comm.*) and may be widespread (Weller and Ornduff 1977).

Any attempt to consider the likely level of geitonogamous self-pollination in a particular plant-pollinator system must include an estimate of within plant visitation along with an estimate of pollen carryover. Up to this point the only attempt to measure both these parameters has been Geber's work on *Mertensia*. She concluded that geitonogamy is not a limiting factor on the reproductive fitness of increasing floral display size. Conclusions drawn by other workers must be treated with caution until further studies are completed.

CHAPTER SIX

MOVEMENT PATTERNS OF POLLINATORS WITHIN PLANTS OF *MYOSOTIS COLENSOI*

It has been frequently suggested that the foraging behaviour of pollinators is be of major importance in shaping the evolution and speciation of plants (Grant and Grant 1968, Faegri and van der Pijl 1971). One aspect of foraging behaviour that has received little attention is the habit of pollinators to move between flowers on the same plant rather than between flowers on different plants resulting in geitonogamous self-pollinations rather than cross-pollinations (see Chapter 5). This behaviour is perhaps not surprising from the point of view of the pollinator, as the behaviour maximises the long term energy gain of its visits (Heinrich and Raven 1972). A pollinator may maximise its efficiency by visiting as many flowers as possible before moving to another plant and thereby minimise the amount of time spent moving between plants (Pyke 1981b). The emphasis on energy-maximising foraging behaviour in recent years, has led to an examination of the behaviour of pollinators and other foraging animals in nature (reviewed in Stephens and Krebs 1986). Behaviour that appears to violate the "rate-maximising rule" is of particular interest. Several recent workers have demonstrated that pollinators frequently leave a plant before all the flowers are visited, contrary to expectations (Zimmerman 1979, Hodges and Miller 1981, Best and Bierzychudek 1982, Geber 1985, Andersson 1988, Schmid-Hempel and Speiser 1988).

Incomplete utilisation of the resource may indeed be optimal, however. Charnov (1976) and Parker and Stuart (1976) simultaneously proposed versions of the "marginal value theorem", which states that an animal should leave a patch when its instantaneous rate of energy gain drops below the average long term rate expected from the habitat. They assume that as an animal forages at a patch, patch depletion occurs, ie the returns per unit time or effort gradually diminish. The reasons for patch depletion are varied (Charnov et al. 1976), but one of the most important may be that as time spent on a patch increases, foragers begin to re-visit previously

worked ground. This type of depletion may cause a forager to leave a patch even though some resources remain unexploited. The idea has been used to explain the observations that some pollinators do not visit all the flowers in an inflorescence (Pyke 1978a, b, Best and Bierzychudek 1982).

Another component of foraging strategies is the relative direction of successive movements. If there is flight directionality, the pattern of correlated movement between successive items, a constant direction is maintained. Such directionality is exhibited by many foraging animals (Levin et al. 1971, Cody 1974, Smith 1974, Pyke 1978c, Waddington 1980). Several authors including Pyke (1978a, c) and Cody (1974) have argued that such directionality is adaptive as it minimises the chance of revisitation. Directionality may be weak, however, when the costs of revisitation is low (Gill and Wolf 1977, Heinrich 1979, Zimmerman 1979, 1981) or when foragers are unable to detect energetic costs of revisitation (Hodges and Miller 1981). All studies on directionality of pollinators, with the exception of Waddington (1980) and studies dealing with vertical inflorescences, have been concerned with the flight movements *between* inflorescences or plants. Waddington, however ((1980), studied honeybees feeding on artificial flowers arranged on a horizontal plane and also found considerable directionality in their movements. Pyke and others (Pyke 1978b, Heinrich 1979, Waddington 1981, Best and Bierzychudek 1982) have studied the directionality of pollinators on vertical inflorescences and concluded that pollinators generally move only upwards and avoid revisits by not circling around the stem. No study has examined directionality between flowers on a plant that presents its flowers in a horizontal plane.

This chapter attempts to (1) confirm by computer simulation, that in a two-dimensional array of flowers a lack of directionality will increase revisitation rates, (2) examine the extent of directionality exhibited by *P.huttoni* foraging at plants of *M.colensoi*, and (3) determine the distance over which movements occur between successive flower visits.

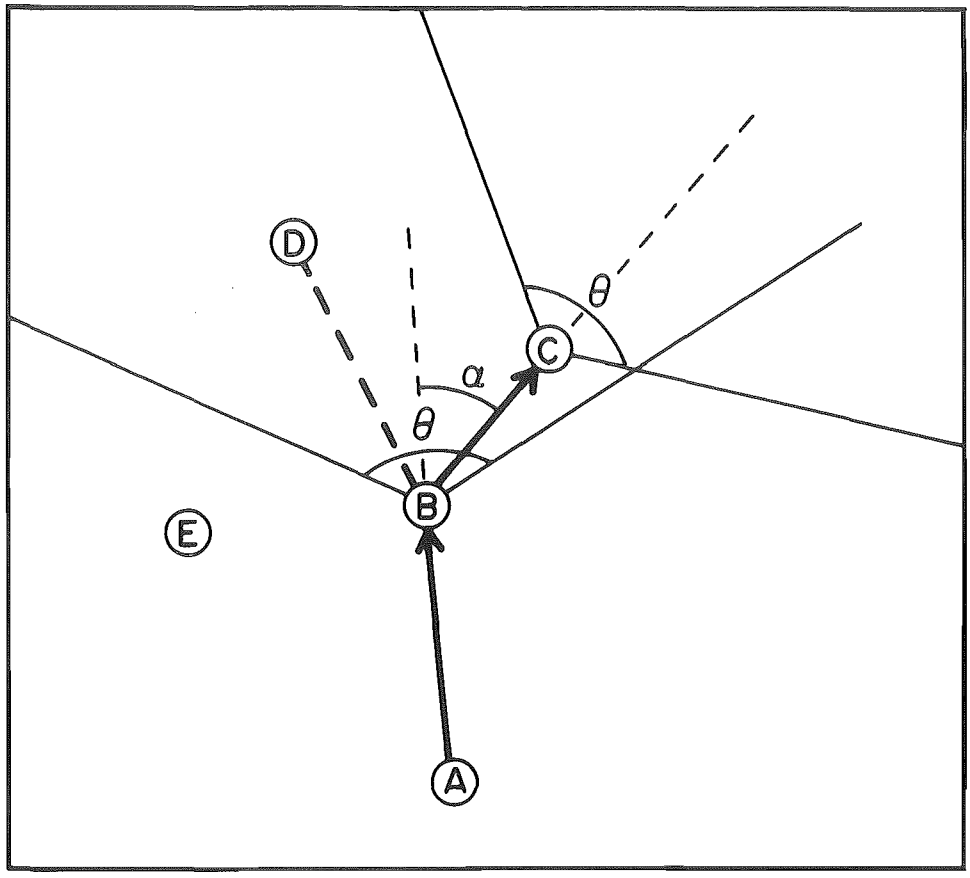


Figure 1. Diagram summarising the movement rules of the computer simulation. A pollinator travels from flower **A** to flower **B**. The line of sight on arrival at **B** is defined by the line connecting **A** with **B**. After collecting the nectar from **B**, a sector Θ is scanned for new flowers. Flowers that fall inside this sector (**C** and **D**) are potential candidates for the next visit. **E** is ignored as it lies outside the search sector. Potential flowers are ranked by their relative closeness. The likelihood of being chosen is a power relation of a flower's proximity relative to that of the nearest flower. Once a flower is chosen, it is directly moved to, visited, and the process is repeated. The angle α is the deviation form straight ahead. A bout ends when the edge of the patch is reached.

METHODS

Computer Simulation

In order to investigate the effects of directionality on the efficiency of foraging strategies, a computer model was developed. The model was based around the following rules (see Figure 1):

(1) Flowers are randomly arranged in a two-dimensional array and contained within a circular "patch" or plant.

(2) Each flower is equally attractive to the pollinators.

(3) A pollinator lands randomly on a flower anywhere within a patch.

(4) Flowers can only be visited if they are contained in a search sector. This sector is defined by line segments radiating out from the flower the pollinator is on and enclosing a variable sector of A degrees either side of the line of sight.

(5) A flower is chosen from within the search sector by selectively weighting flowers according to their proximity. All flowers are ranked in order of proximity. The relative probability of a particular flower being visited next is found by the ratio of its distance away to that of the nearest flower raised to a particular power. For the purposes of the simulation below the exponent was set at 9. The relative chance of a flower being visited next is weighted by this ratio:

$$(n/d)^9$$

where n = the distance to the nearest flower and d = the distance to the flower in question. A flower from the search sector is thus chosen randomly using this weighting constraint.

(6) Visits to successive flowers continue until the edge of the patch is reached and no flower is present satisfies the criterion defined in (5). When this happens the bout is over.

A computer program was written in BASIC using these rules. 50 runs of pollinators, each foraging on a new randomly chosen array of 50 flowers, were carried out for each of the following search sectors: 20, 40, 60, 80, 100, 120, 140 and 160 degrees. For each run, the number of flowers visited and the number of these visits that were re-visits were recorded.

Foraging behaviour of P.huttoni

The degree of directionality exhibited by *P.huttoni* foraging on plants of *M.colensoi* was examined with the aid of video photography. On several days in November 1988, large plants were chosen to film. A video camera was set up on a tripod directly above a plant at a height of about 1.5 meters. The camera was set up to record continuously over the course of several hours, beginning usually around 10.00 a.m. before pollinator activity had begun until late in the afternoon. The camera was approached only to change tapes and check the operation. The resulting film gave a record of the visitation episodes for a whole day. During the day, a still

photograph was taken to be used later to produce a map of the flowers for recording pollinator paths onto later (Figure 2). The tapes were later viewed, and the pieces of tape where a fly was recorded visiting the plant were dubbed to another tape for later detailed viewing.

Subsequently, the tapes were viewed and the exact path a pollinator took was recorded on the map produced from the still photograph. The angle from the line of sight defined by the line of travel from the previous flower was measured and the distance of each move was recorded. Moves were distinguished as walks, where the fly crawled across the patch from one flower to another, or short flights, where the fly actually flew across the patch to reach the next flower.

RESULTS

Computer simulation.

Figure 3 shows the results of the model in which directionality of movement was varied. Figure 3(a) shows the percentage of visits that are revisits to flowers that had been previously revisited on that bout. As the size of the search sector increases beyond about 120 degrees, i.e. 60 degrees either side of the line of sight, the proportion of revisits dramatically increases. With a search sector of 160 degrees, almost 40 % of the flowers visited in the simulation had already been visited.

Figure 3(b) predicts the energetic effect of varying the search sector. As the "cost" of revisiting a flower is unknown, the relationship is shown for various postulated levels of cost varying between zero and one. A zero cost assumes that no energy is lost by wasting time visiting a previously visited flower. A cost of 1.0 assumes that visiting a previously visited flower causes a loss of the same amount of energy that is gained from visiting a previously unvisited flower. The energy gain is the total amount of energy gained in one bout. As such, it depends on the number of previously unvisited flowers that are visited in a bout. Remember that for this simulation, a bout ends when the edge of a patch is encountered.

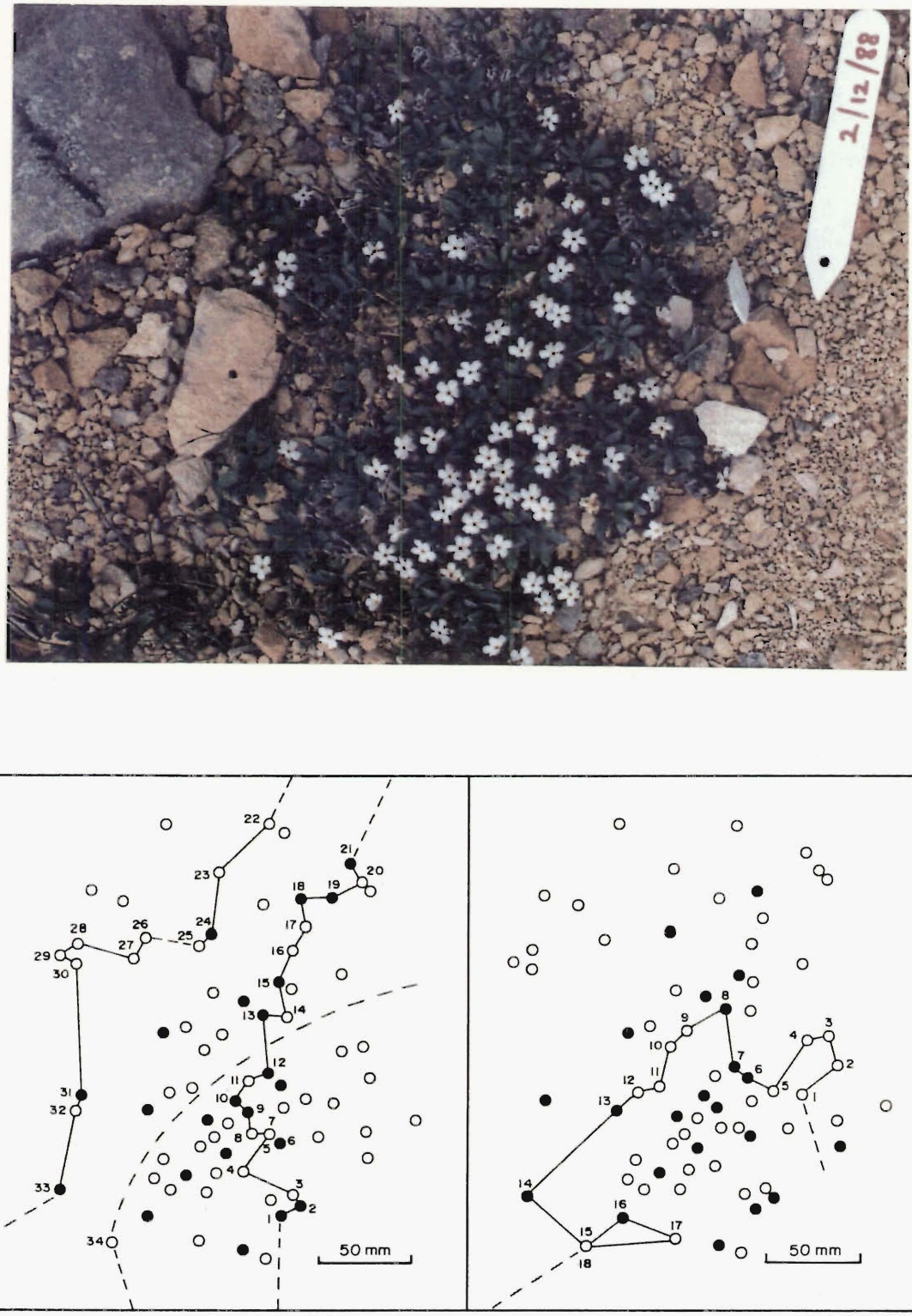


Figure 2. A representative plant and two observed bouts. The positions of flowers were traced directly off the photograph. The filled circles represent flowers that are in the presentation-phase while empty circles represent the post-presentation phase. The numbers represent the order in which flowers were visited. Walks are shown as solid lines and flights as dashed lines.

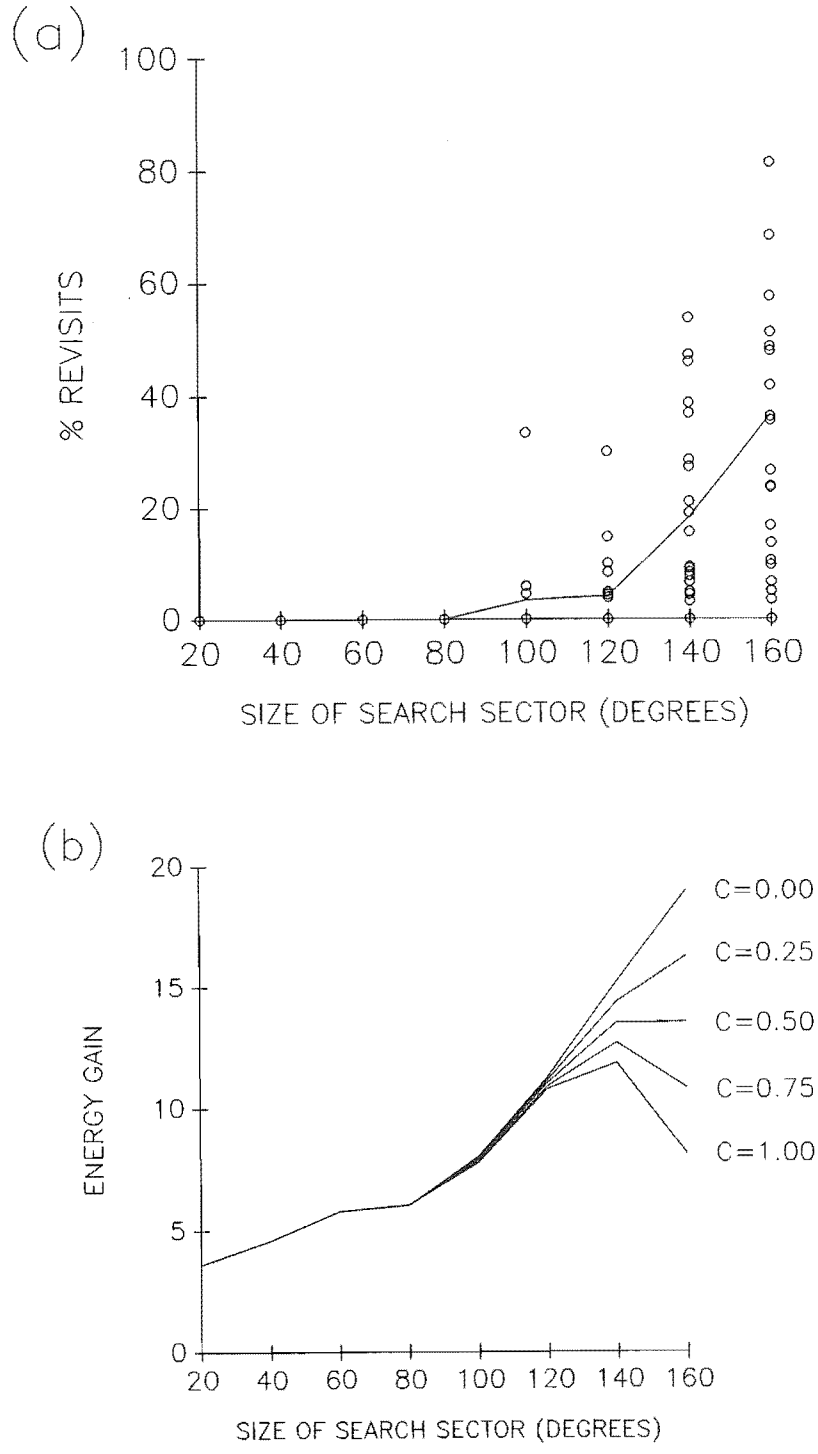


Figure 3. The costs of widening the sector size.

a. The relationship between the size of the search sector and the percentage of visits that are revisits.

b. The proposed cost of revisits. Energy gain is directly proportional to the number of previously unvisited flowers that are visited on a bout. Various costs (**C**) are imposed as a result of revisiting a previously visited flower. **C** ranges from 0 cost (no energy is lost by the time spent probing an empty flower) to a cost of 1.0 (as much energy is lost as is gained from visiting a flower that has been previously visited during this bout).

In real bouts, however, it was observed that P.huttoni often turned around when it reached the edge of a patch (see below), so that the total number of flowers visited in a real bout may not alter as the search sector changes. Nevertheless, the distance moved (and therefore the time used) between flowers will vary in proportion to the number of flowers visited in the simulation, because a fly must move further to find flowers if the search sector is narrowed. If the assumptions made here are satisfied and there is at least a moderate cost of revisiting flowers, there appears to be an optimum search sector of around 140 degrees. A narrower sector would reduce revisitation but require larger distances to be moved, while a wider sector would lead to more revisitation but shorter movements.

Behaviour of P.huttoni

Figure 4 shows the scatter of movements made by *P.huttoni* on plants of *M.colensoi*. The movements shown are relative to the axis of travel from the previous flower. The distance from the center of the plot is proportional to the actual distances moved. Figure 4(a) shows walking movements while Figure 4(b) shows the much less common short flights. The walks show a relatively high degree of directionality while the flights appear to be essentially random.

Figure 5 presents the distributions of deviations from -180 degrees (anti-clockwise backwards) to 180 degrees (clock-wise backwards). Batschelet (Batschelet 1965) describes the properties of circular distributions and statistical methods of analysis of such distributions. His recommendations are followed here. Figure 5(a) shows the distribution of walks which are clearly unimodal with a mean angle that is not significantly different from 0 degrees (Stephen's exact test, mean angle = 2.33 degrees). Although the distribution is not adequately fitted by a circular-normal distribution (X^2= 49.62 0.01> P > 0.0001) it is reasonably close and may usefully summarise the distribution.

The shape of the distribution may also be described by the mean angular deviation (M.A.D.)(Waddington 1980). The M.A.D. acts in a similar way to the standard deviation of the normal distribution. Thus the M.A.D. contains 67% of the distribution. The larger the M.A.D., the

more frequent large changes in direction become. A M.A.D. of 81.0 degrees would indicate that direction is random with respect direction (Batschelet 1965). The M.A.D. for walks = 56.1 degrees, which clearly differs considerably from random. Directionality may also be considered as the average vector component of successive movements in the forward direction (Levin et al. 1971, Pyke 1978a). An average vector of 1.0 would indicate perfect directionality while an average vector of 0 would indicate randomness with respect direction. The average forward vector for walks was found to be 0.52. On all measures, the walks seem to show a moderate level of directionality. They do not, however, appear to be restricted to a search sector of 140 degrees as postulated to be the optimum in the previous section.

On the other hand the flights appear to be essentially random (Figure 5(a)). However, there is a significant deviation from a uniform distribution (X^2 = 11.9, P > 0.05). Despite this, there is no indication of a significant central tendency. k, a measure of central mass is not significantly different from 0, (Raleighs test, Batschelet 1965). The M.A.D. is equal to 72.62 degrees and the average forward vector is equal to 0.196. Thus, there appears to be very little directionality to the short flights.

Directionality may also be achieved by alternating left and right turns (Pyke 1978c). Pyke (1978c) tests this by comparing the frequencies of left turns followed by right turns and vice-versa. However, this test would pick up significance only if the deflection was compensated directly in the next move. If the compensation was delayed by one or more moves it would not be picked up by this test. A better test may be the "runs test". This test checks for the number of runs or trends in either of two directions, in this case, movements left or right. Significance may result from either too many short runs (a significant alternation of direction) or by too few (a tendency to circle by making moves in one direction either circling left or right). Table 1 presents the results of this test on bouts where 10 or more flowers were visited. In all but a few cases, there is no trend in the runs. Out of 28 tests three indicate too many runs but 1 the reverse. Hence there is no consistent evidence that *P.huttoni* alternates left and right turns or moves in a circle (which would reduce directionality).

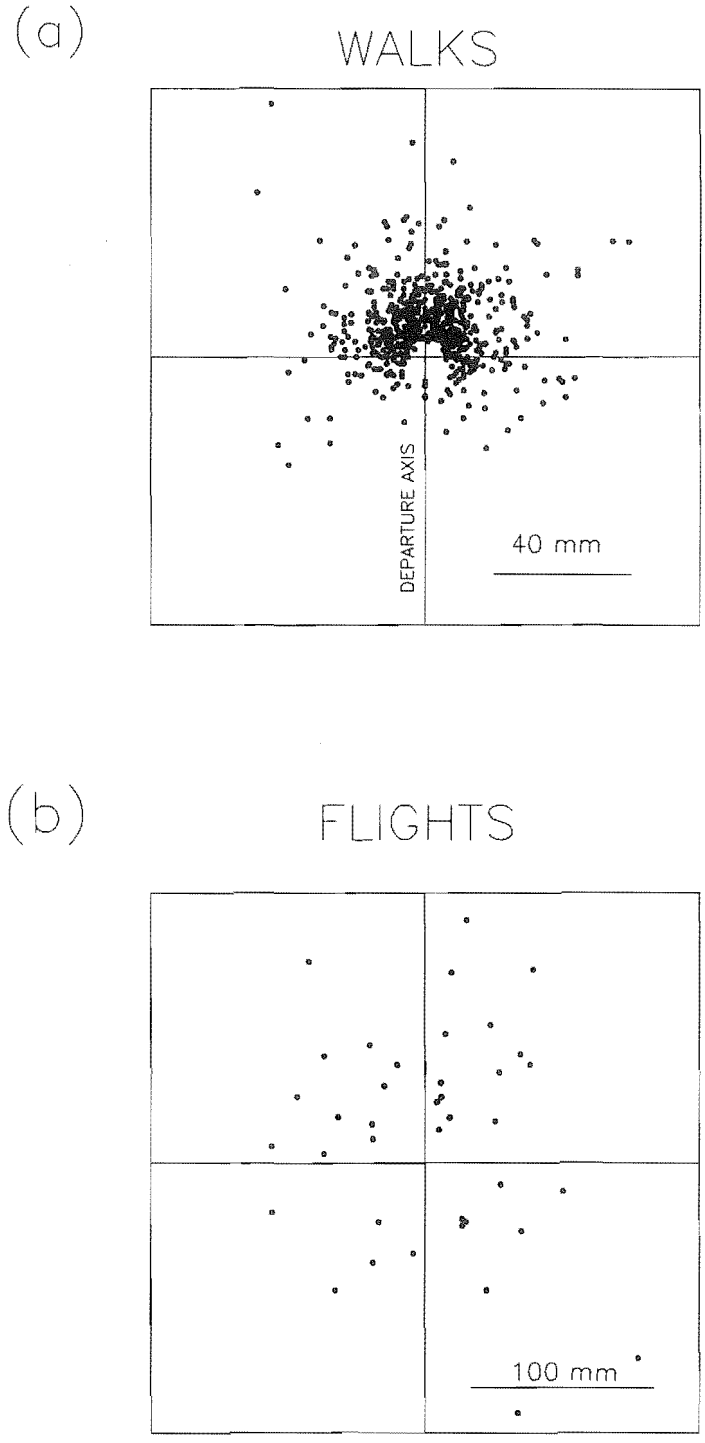


Figure 4. Movements in the two travel modes relative to the departure axis from the previous flower.

a. Scatter plot of walks.

b. Scatter plot of short flights.

The frequency distributions of movement distances are shown in Figure 6. The distribution for walks (Figure 6(a)) is very strongly leptokurtic, indicating that most movements are very short (median = 14.4 mm). The first size class has less visits because flowers must be at least 7 mm apart (the diameter of the flower itself). There seems to be a strong preference for visiting flowers that are near neighbour. This largely justifies the strength of the weighting on "nearness" used in the computer simulation above. The short flights are much more erratic however and less leptokurtic. They also tend to involve much larger distances (median = 63.0 mm).

As a result of the tendency to maintain directionality between flowers, the frequency of revisitation is low. Of the 724 walking movements recorded in total, only 41 were revisits (less than 6% of the total).

To summarise, *P.huttoni* utilises two modes of travel within plants of *M.colensoi*. Walking over short distances is the usual mode of movement but it is occasionally interrupted by short flights from one part of a plant to another. The two modes differ in their degree of directionality. Walks are usually made in a way that maintains a forward movement across the plant. Short flights however, are essentially random with respect to direction. In addition, walks are usually very short with a strongly leptokurtic distribution, while the flights are over longer and more variable distances. There was no tendency to alternate left and right turns in either mode.

DISCUSSION

Despite the considerable degree of directionality demonstrated by *P.huttoni,* it is apparently less than the optimum amount judged by the results of a computer simulation. A search sector of 140 degrees appears to be ideal, but 27.2% of the walks involve greater change directions than this. It would appear that the costs of sharp changes in direction are less than the benefit of shorter travel distances and/or greater residence time in profitable sections of a plant (see below).

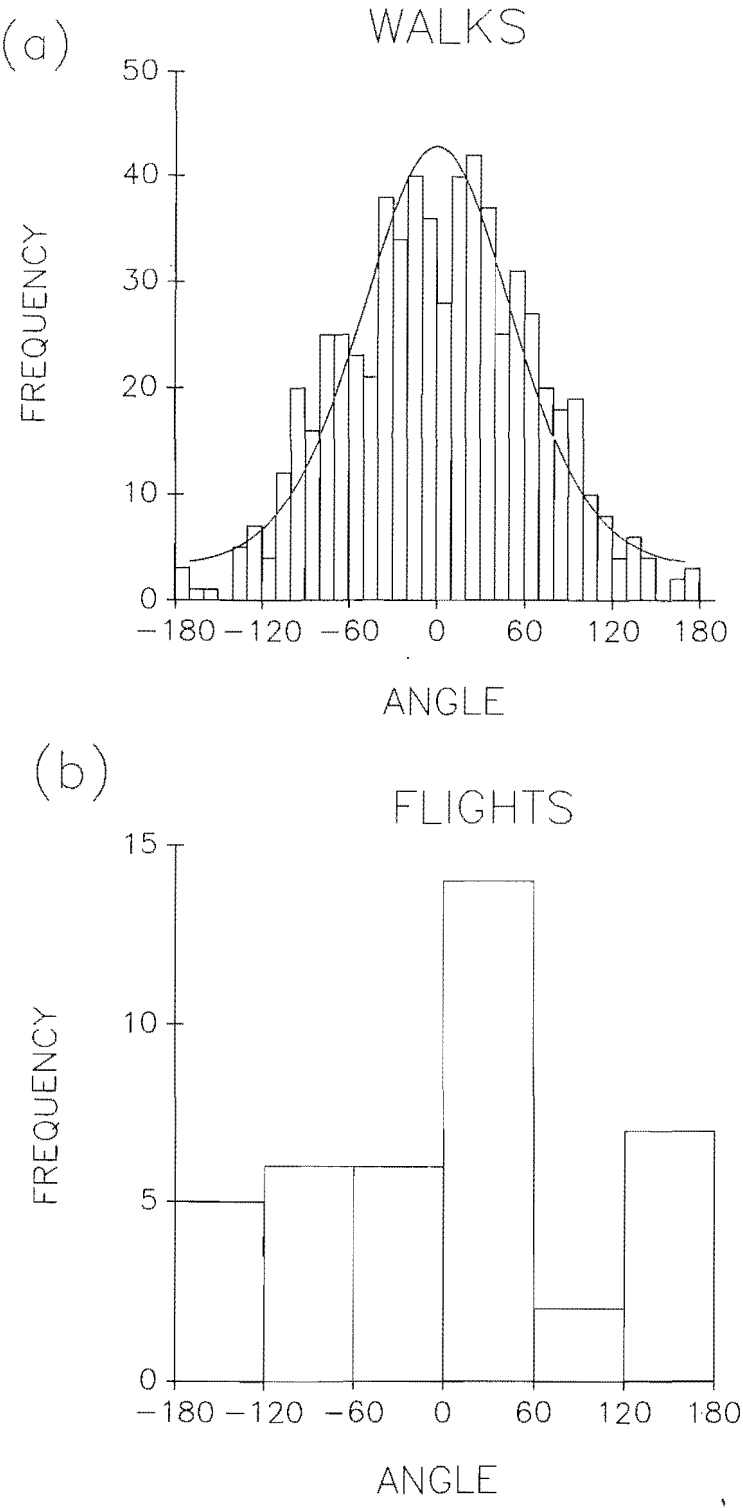


Figure 5. The distribution of movement directions in the two travel modes.

a. The distribution of the walks. A circular-normal distribution is shown fitted to the data.

b. The distribution of the short flights. There is no indication of departure from a uniform distribution.

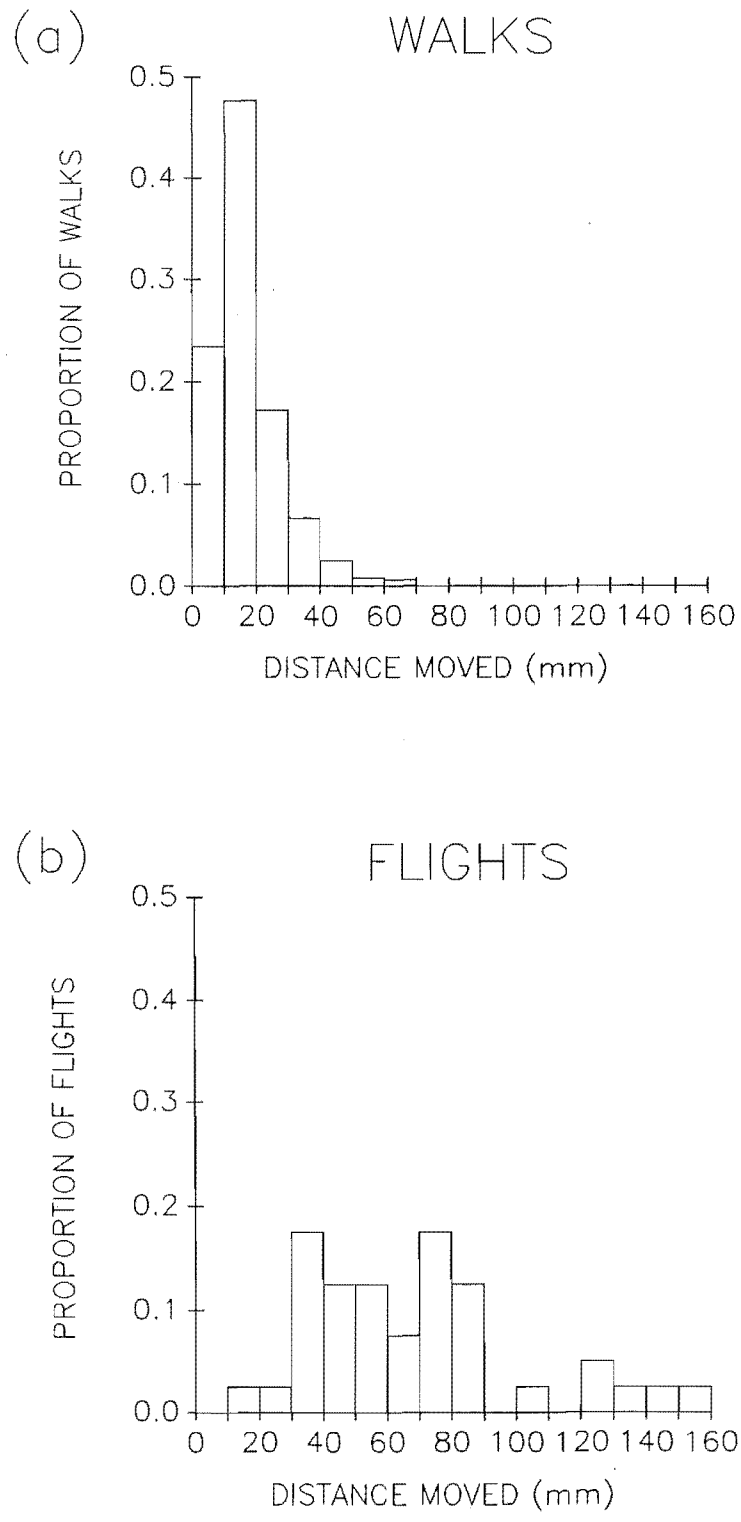


Figure 6. The distribution of the lengths of movements for the two travel modes.
a. Walks.
b. Short flights.

Table 1. The observed and expected number of "runs" of left and right turns.

SEQUENCE	NO. FLOWERS VISITED	NO. RUNS	EXPECTED NO. RUNS	+/-*
96	11	8	6.5	
415	39	22	25.7	
501	15	9	9	
565	10	3	5.5	
810	11	6	6.5	
909	49	28	32.3	
1291	10	4	5.5	
1488	22	12	13.5	
1528	31	13	20.3	-
1697	14	6	8.5	
2032	13	7	8	
2056	22	13	13.5	
2116	10	9	5.5	+
2121	18	10	11	
2368	22	13	13.5	
2439	11	9	6.5	
2630	11	5	6.5	
2712	32	19	21	
2903	20	12	12.5	
2975	30	14	19.7	
3037	10	9	5.5	+
3054	66	43	43	
3162	32	24	21	
3242	17	8	10.5	
3412	18	16	11	+
3548	13	6	8	
3631	27	17	17.7	

* A plus or minus sign in this column indicates sigificantly (at the 5% level) more or less runs than expected according to the runs test.

A similar result was found by Pyke (1978a) who simulated the foraging behaviour of pollinators on a regular array of varying size. He fixed the number of flowers to be visited in each bout and assumed that patch boundaries reflect the pollinator back into the patch. The pollinator was

restricted to moving to flowers that are directly ahead, directly behind or to either side of the present flower. The probability of each of these four types of movement was allowed to vary and sets the level of directionality. This model is very similar to one used by Cody to simulate the foraging strategies of bird flocks (1971). The model used here differs in several respects. Firstly, flowers were arranged at random within a circular plant rather than in a regular array as in Pyke's model. Movements were possible to all flowers within the search sector rather than only to four neighbours. The probability of visiting a particular flower was fixed by its relative proximity and was not affected by its direction as long as it was in the search sector. This differed from Pyke's model where flower probabilities were assigned to each of the 4 possibilities by the direction. Despite these differences, the two simulations agree that pollinators should display considerable directionality in order to minimise revisitation.

The amount of directionality found here is similar to the findings of previous measures of pollinator foraging behaviour *between* plants. The following vector directionalities have been found: 0.39 for bees and 0.44 for butterflies (Levin et al. 1971); 0.46 and 0.57 for bumble bees (Pyke 1978a), compared with 0.52 this study. Waddington (1979, 1980) found mean angular deviations of between 56 degrees and 67 degrees for honeybees and sweat bees. However, several other recent studies have revealed a lack of any directionality in a variety of animals collecting pollen or nectar (Gill and Wolf 1977, Zimmerman 1979, 1981, Pyke 1981c, Hodges and Miller 1981). Zimmerman (1979) suggested that such a lack of directionality might be in fact optimal where the proportion of flowers probed on a plant was small and therefore the probability of retracking causing revisitation is slight. Waser (1983) reviews the evidence and concludes that directionality does indeed seem to be more important where the proportion of flowers visited in a bout is high. In the case considered here, the patch is essentially a single plant. Thus the flowers may be considered to be equivalent to the "plants", and a revisit means a second visit to a particular flower and will be strongly penalised. Thus Zimmerman's argument cannot be applied here. Hodges and Miller (1981 concluded that the lack of directionality they found may have been caused by the pollinator being unable to detect the cost in terms of reduced reward (pollen) from revisiting a patch. It seems unlikely that *P.huttoni* is unable to perceive this cost as nectar is

actively collected.

Pyke (1978a) suggested that the lack of strong directionality may be caused by a patchy distribution of food. He suggested that less directionality may occur when particularly good patches are found. Indeed some evidence has been provided by other work. Pyke (Pyke 1978c) showed that the M.A.D. was higher when more flowers were visited on an inflorescence (but see Waddington and Heinrich 1981 who suggested that Pyke's result may be due to the insects losing their orientation with respect to previous movements as more flowers are visited). Heinrich (1979 showed that bees made more direction changes in nectar-rich patches than they did in depleted patches. Waddington (1980) however, found no such pattern. It should be possible to test whether longer foraging time (and hence greater reward recovery) in *P.huttoni* influences directionality within a plant. This will be attempted subsequently.

There is no evidence in this study that left and right turns are alternated as found by Pyke (Pyke 1978c). Zimmerman (1982) also failed to find such a behaviour.

The comments of Waddington and Heinrich Waddington and Heinrich 1981 are a reminder of the caution required in interpreting behaviour as always being adaptive. In particular, although the data presented here seem consistent with an hypothesis that *P.huttoni* is attempting to minimise revisitation, it is possible that other explanations exist. For instance, the characteristically forward movements may be simply be the result of the relative ease of moving forward as opposed to reversing direction. It will be difficult to prove that the correlation between successive directions is a conscious effort on the part of the pollinator to reduce visitation. A demonstration that insects will reverse directionality when conditions are good will at least indicate that they are least potentially capable and willing to alter directionality. If this can be shown, there may be a stronger case for suggesting that directionality in the normal case is adaptive.

The distances moved between flowers on walks are highly leptokurtic. Leptokurtic

distributions are frequently found for between-plant flights (review in Waddington 1983). Such patterns are thought to minimise the energy spent in foraging. Some observations have suggested that like directionality, flight distances may be influenced by reward rate. Pyke (1978c) found interplant distances were greater after "poor" plants had been visited. Heinrich (Heinrich 1979) and Waddington (Waddington 1981) have found similar results. The short flights exhibited by *P.huttoni* may be acting in a similar way as an attempt to reach better flowers. Again, it should be possible to test whether there is any association between the rewards recovered and the distance moved and the frequency of short flights and is contemplated in the future.

This chapter does not consider leaving rules. It is of considerable importance in terms of the frequency of geitonogamy, to understand what causes pollinators to leave a plant. The usual theoretical models have emphasised the marginal value theorem and assume that foragers make decisions based on their "instantaneous gain functions". Other work, has shown that such a rule is inefficient whenever patch assessment is important (McNamara 1982, Stephens and Krebs 1986. Patch assessment may be important whenever resources become distributed patchily. Such patchy distributions of nectar are likely to be imparted by the foraging of the pollinators themselves. The data available from the video tapes will be examined to determine whether a simple departure rule exists.

In summary, *P.huttoni* appears to demonstrate a moderately high level of directionality movements between flowers on plants of *M.colensoi*. This directionality appears to minimise the frequency of revisits. However, changes of direction are found and sometimes lead to revisitation. They may be a mechanism to remain in a favourable plant or group of flowers. Short flights sometime occur within a patch and may be an attempt to reach more favourable flowers. Such flights are random with respect direction and variable in length. The more usual walking mode of movement is much more restricted in distance and may minimise the time spent travelling within a patch. Other data is desirable, particularly relating the apparent rewards gained from flowers to directionality, move lengths, the frequency of short flights and the factors

that control the decision to leave a plant. Some caution may be required however, in order to avoid the danger of interpreting all aspects of behaviour as adaptive. Other simple explanations for observed behaviour may also be involved that do not assume energy gain is maximised.

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